

PATHOLOGICAL AND THERAPEUTIC INVESTIGATIONS  
IN TUBERCULOSIS

PART I    PATHOLOGICAL INVESTIGATION

An Investigation into Hypersensitivity  
in Tuberculosis as illustrated by the Response  
of the Skin, and of the Leucocytes of the  
Blood to Tuberculin.

PART II    THERAPEUTIC INVESTIGATION

A Clinical Evaluation of the Use of  
para-Aminosalicylic Acid in the Treatment  
of Pulmonary Tuberculosis.



a thesis

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PART I

PATHOLOGICAL INVESTIGATION

AN INVESTIGATION INTO HYPERSENSITIVITY  
IN TUBERCULOSIS AS ILLUSTRATED BY THE  
RESPONSE OF THE SKIN, AND OF THE LEUCOCYTES  
OF THE BLOOD TO TUBERCULIN.

## Chapter I

### INTRODUCTION

This investigation owes its inception to the discussion by the Tuberculosis Society of Scotland of a paper by Clayson (1940). This writer examined the relationship of skin sensitivity to tuberculin in experimental animals to the progress of the tuberculosis in these animals. He suggested that caseation and liquefaction took place most rapidly in those animals showing a high degree of skin sensitivity, while fibrosis took place most readily in those demonstrating a low degree of skin sensitivity. It was also found, however, that in the animals with a high degree of sensitivity a more effective localisation of the infection took place. It is unnecessary to examine in detail these two apparently, in part at least, contradictory statements. But in the discussion which followed the presentation of the paper, Cameron (now Professor Cameron) suggested that no complete proof was available to show that skin sensitivity per se necessarily represented the genuine sensitivity of other body tissues. Subsequently Cameron (1946) has described a patient in whom, despite presumptive evidence of the presence of an active tuberculous focus, no response of the skin to tuberculin, including a dilution of 1:10, could be obtained. A response, however, similar in/

in nature to that obtained in tuberculin sensitive individuals, was demonstrated on vaccination with the vole bacillus. This latter phenomenon, simulating as it did the altered response of tissues sensitised to the products of the tubercle bacillus yet without, in the same subject, demonstrable sensitivity of the skin to tuberculin, has also been described by workers investigating the response of the tissues to Bacillus-Calmette-Guérin (Ustvedt 1949). This raises issues of great importance in assessing tuberculin hypersensitiveness and its place in the pathogenesis of tuberculosis.

Further investigation into this problem was needed and the purpose of this thesis is to present and analyse evidence of tuberculin sensitivity as observed in the tissue (other than the skin) most readily available for examination, namely the blood.

#### ACKNOWLEDGEMENTS

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I am also indebted to Professor Kermack, Royal College of Physicians Laboratory, Edinburgh, who supplied the tuberculin which was used throughout this investigation.

OBJECT:/

OBJECT: Three main objects may be generally stated as forming the basis for this inquiry.

To determine:

- (i) Whether or not an altered response to tuberculin could be demonstrated in the leucocytes of patients suffering from tuberculosis.
- (ii) The nature of this response, if so demonstrable.
- (iii) The relationship, if any, of this response to the manifestation of hypersensitivity produced in the skin by the intracutaneous introduction of tuberculin.



Chapter 2REVIEW OF LITERATURETuberculin

Since the isolation by Koch in 1890 of a substance liberated by the tubercle bacillus during its growth in culture and which he termed tuberculin, many workers have sought to determine the relationship this substance bears to the pathological action of the tubercle bacillus in the animal body.

Nature of Tuberculin

The material generally known as tuberculin (T), or sometimes inaccurately referred to as Koch's Old Tuberculin (OT), is the fluid medium in which tubercle bacilli have been grown, sterilised by heat, freed from bacillary bodies by filtration, and concentrated by evaporation to one tenth of its original bulk.

The conclusion arrived at by Koch that the active principle of tuberculin was protein in nature has been confirmed repeatedly by the studies of subsequent investigators, notably those of Long and Seibert (1926). These workers proved conclusively that the active substance in tuberculin was tuberculoprotein, and that it produces the same reactions in hypersensitive tissue as the parent substance tuberculin.

Sensitivity in Tuberculosis

When the tubercle bacillus gains access to the body, changes occur in the defence mechanism which manifest themselves in an altered response of the cells/

cells to further contact with the bacillus, and indicate the development of hypersensitivity to the bacillus. (Note:- The terms altered response, sensitivity and hypersensitivity will be used synonymously in this paper to indicate the response of sensitised tissues to the sensitising antigen, in this case tuberculoprotein or tuberculin.) These are the changes underlying the "Koch phenomenon" and described by him in the following words:- "If a normal guinea pig is inoculated with a pure culture of tubercle bacilli, the wound, as a rule, closes and in the first few days seemingly heals. After ten to fourteen days, however, there appears a firm nodule which soon opens, forming an ulcer that persists until the animal dies. Quite different is the result if a tuberculous guinea pig is inoculated with tubercle bacilli. For this purpose it is best to use animals that have been infected four to six weeks previously. In such an animal, also, the little inoculation wound closes at first, but in this case no nodule is formed. On the next or second day, however, a peculiar change occurs at the inoculation site. The area becomes indurated and assumes a dark colour, and these changes do not remain limited to the inoculation point but spread to involve an area 0.5 to 1.0 cm. in diameter. In the succeeding days it becomes evident that the altered skin is necrotic. It finally sloughs, leaving a shallow ulcer which usually heals quickly and/

and permanently, and the regional lymph nodes do not become infected. The action of tubercle bacilli upon the skin of a normal guinea pig is thus entirely different from their action upon the skin of a tuberculous one. This striking effect is produced not only by living tubercle bacilli, but also by dead bacilli, whether killed by prolonged low temperature, by boiling or by certain chemicals."

The mechanism which causes these changes is still obscure, but, that it would appear to be connected with the protein constituent of the bacillus, is indicated by the fact that under experimental conditions the power of inciting this altered response of the tissues is possessed by the protein constituent (Sabin 1941). This property is, however, greatly enhanced by the addition of the tuberculo-phosphatide fraction of the bacillus. Two facts have emerged, however, which indicate that this theory does not wholly explain the phenomenon of sensitivity in tuberculosis as manifest in the animal body. Firstly, it is the belief of Corper (1946) following extensive studies into the mechanism of specific tuberculo-immunity in tuberculosis that tuberculoprotein is not liberated in appreciable quantities by the tubercle bacillus in vivo. Secondly, the artificially induced hypersensitivity mentioned above differs in one important feature from that produced by the bacillus either in the viable or non-viable state. This difference consists in/

in absence of response to the heat treated product "Old Tuberculin" when this substance is injected into the skin although the typical skin response is obtained when the sensitising antigen, tuberculo-protein, is used.

#### "Tuberculin type" reaction

Despite this uncertainty as to the exact mechanism of production, the response to tuberculin of the skin of an individual previously infected with the tubercle bacillus is a clear cut phenomenon and has long been utilised as an ancillary procedure in the diagnosis of tuberculosis. It is a local manifestation of the altered response of the tissues known as the "tuberculin type" reaction and is characterised by the appearance, only after some hours, at the site of injection of the antigen (tuberculin), of an inflammatory reaction reaching its maximum within 24 - 48 hours.

This response of the skin provides an easily measurable and readily accessible manifestation of the reaction of one type of tissue in the body.

#### Focal reaction

That the cells of other tissues in the sensitised body exhibit an altered response to this antigen would seem to be indicated by the "focal reaction" which takes place at the site of a tuberculous lesion when tuberculo-protein reaches it by way of the blood stream. This focal reaction consists of hyperaemia sometimes with necrosis and haemorrhage/



haemorrhage and this seems related to the phenomenon described and investigated, amongst others, by Shwartzman. In the "Shwartzman phenomenon" haemorrhage and necrosis take place at the site of lesions caused by different injurious agents or bacilli when the appropriate antigen reaches them via the blood stream. This phenomenon is thought to depend on the enhanced vulnerability of the capillaries at the site of the original lesion.

There is, however, no proof that the tissues at the site of a lesion exhibit a greater hypersensitivity than those elsewhere in the body. Indeed it might be argued that in progressive tuberculosis the tissue at the site of a lesion is likely to be less hypersensitive owing to the continued liberation of tuberculoprotein from the large numbers of bacilli in the lesion (Rich 1944). That continued exposure to small quantities of tuberculoprotein does lead to a degree of desensitisation has been shown by Rich (1944) and others in animal experiments and is the basis of treatment with tuberculin, a method at first assayed with great enthusiasm and now found to have only a restricted field of usefulness in the treatment of tuberculosis.

#### Tissue culture studies

In tissue culture studies on experimental animals carried out by a number of workers the response of a wide variety of tissue exposed to the action of tuberculin has been investigated e.g. explants/

explants of spleen and buffy coat of blood were used by Rich and Lewis (1932): explants of spleen, lymph node and buffy coat of blood by Heilman and Feldman (1944): a mononuclear exudate suspension obtained by the intrapleural injection of molten parowax and explants of spleen by J.K. Moen (1936): explants of bone marrow, spleen and testes by J.D. Aronson (1931, 1933); to mention a few.

#### Cytotoxic action of tuberculin

These studies have proved conclusively (i) that the cells of tuberculous animals are injured by concentrations of tuberculin which do not affect the cells from non-tuberculous or normal animals: (ii) the cells retain this hypersensitivity when isolated from the body in tissue culture and it persists after several transplantations in vitro: (iii) this sensitivity of the cells is exhibited even in the absence of plasma or serum and is therefore an inherent characteristic of the cell which cannot be passively transferred: (iv) the presence of the serum from tuberculous animals in the cultures of normal cells produces no toxic effect on addition of tuberculin, therefore circulating antibody is not necessary for the allergic damage to occur and no toxic substance is produced by the admixture of antibody and antigen. Rich postulates that the vital antibody is bound directly to the cell.

Similarly, further exhaustive studies into the exact nature of the cytotoxic action of tuberculin have/



have shown (i) that coagulable proteins behave differently to bacterial proteins when added to tissue cultures obtained from the respective sensitive animals, i.e. in the anaphylactic type of hypersensitivity induced by these proteins the cells of the sensitised body are not susceptible in tissue culture to the appropriate antigen. This has been demonstrated for such proteins as horse serum, egg albumen and beef lens (Aronson and Moen, 1933). It has also been shown that concentrated glycerol broth prepared as for tuberculin has no cytotoxic action on cells showing tuberculin hypersensitivity (Aronson, 1931), (Moen and Swift, 1936).

(ii) That "tuberculin" prepared from other acid fast bacilli and mammalian tubercle bacilli in a similar manner to Old Tuberculin has no action on tuberculin-sensitive cells (Aronson, 1931).

(iii) That the specific cytotoxic action of tuberculin is also demonstrable in tissue cultures from animals sensitised with heat-killed tubercle bacilli (Heilman, Feldman and Mann, 1945).

(iv) That purified protein derivative (P.P.D.) of tuberculoprotein has the same cytotoxic action on sensitised cells as tuberculin (Heilman and Seibert, 1946).

#### Measurement of cytotoxic action

These conclusions were based mainly on retardation of growth and the inhibition of migration of the sensitive cells when incubated with tuberculin in vitro and a measure of the cytotoxic action/

action of tuberculin in a particular subject has been elaborated. It is known as the comparative cytotoxic index and can be represented thus:-

$$\frac{\text{Average migration (normal cells)}}{\text{Average migration (normal cells + tuberculin)}} \times \frac{\text{Average migration (sensitised cells + tuberculin)}}{\text{Average migration (sensitised cells)}}$$

Corper et al (1945) have also established and measured the depression of phagocytic activity of the cells of tuberculous guinea pigs produced by tuberculin, but no corresponding depression is produced on the cells of normal animals.

#### Systemic and local sensitivity compared

Further to the above, Heilman and Feldman (1946) have demonstrated that in experimental animals loss of skin sensitivity in tuberculous animals, due either to intercurrent infection or in the terminal stages of the disease, was not accompanied by loss of systemic sensitivity as demonstrated by the action of tuberculin in tissue culture. Aronson (1931) had inferred a similar conclusion, namely of the lack of correlation between local and systemic sensitivity in young guinea pigs, after demonstrating by tissue culture that there was no difference between the sensitivity of the tissues of young guinea pigs and those of mature animals, while Freund (1929) had shown that the skin reactions of young guinea pigs infected with tubercle bacilli were negative. These last guinea pigs, however, did react with an intensity equal to that of adult animals to the intraperitoneal injection of tuberculin.

Response/

## Response of the skin to tuberculin

In the course of many investigations into the nature of skin sensitivity to tuberculin in the sensitised subject, the occurrence of wide fluctuations in the degree of skin response has been clearly established. Forbes (1938) conducted an interesting investigation in which serial testing was carried out in groups of sensitised individuals not suffering from demonstrable disease. Retesting was carried out at intervals varying from one to six weeks and the results showed that after, in some cases, an initial rise in the size of the reaction, marked fluctuation took place in the degree of the response. Bluhm (1948) has also demonstrated fluctuations in the skin response to tuberculin during the course of the disease in patients suffering from pulmonary tuberculosis, and the diminution in skin sensitivity in the later stages of the disease in miliary and in meningeal tuberculosis has been repeatedly described. (Woodruff, 1946, Pilcher, 1930, etc.). Pilcher appears to have established that the diminution in the response in the emaciated patient in the terminal stages of the disease is due, in part at least, to diminution in the circulation of the skin as he has shown that, in these patients, the response to a non-specific irritant (codein) is also markedly reduced. But in the patient who died before marked wasting had occurred, the same diminution in skin sensitivity was not noted.

Conversely, a diminution in and loss of skin sensitivity/

sensitivity has been demonstrated in serial testing over a period in tuberculin sensitive individuals who may or may not have manifest signs of tuberculous disease. An investigation of this kind was carried out by Puffer et al (1946) who demonstrated the loss of skin sensitivity over a period in school children. Paretzky (1938) and Dahlstrom (1940) also demonstrated the disappearance of skin sensitivity over a period and Paretzky regarded this as a possible manifestation of immunity in that it occurred in some cases where known contact with open cases of tuberculosis was still continued. Whether or not the complete loss of skin sensitivity can be taken as an indication of a healed lesion has not yet been clearly established.

Recent work by Sarber (1948) has shown that diminution of the skin response to tuberculin in tuberculous guinea pigs can be produced by the antihistamine drug, benadryl, thus suggesting that part, at least, of the response in the skin is a demonstration of the reaction of the individual skin cells.

#### Summary

To summarise, therefore, there are several undisputed facts.

(1) Infection with the tubercle bacillus produces in the animal body, mainly, it would appear, by virtue of its protein constituent, an altered response of the tissues which is manifest on subsequent/



quent contact with the bacillus or with tuberculo-protein.

(2) This "altered response" can be demonstrated in tissue cells by means of tissue culture where it is seen that the action of tuberculin on the sensitised cells is toxic in nature.

(3) The "altered response" is manifest in the skin by the familiar delayed inflammatory reaction.

(4) Considerable fluctuation in the degree of the response of the skin can be demonstrated in a sensitised individual.

(5) Substances producing diminution in skin response do not necessarily reduce the susceptibility of the cells of other tissues to the toxic action of tuberculin.

The importance of determining further the relationship of the skin sensitivity to tuberculin to the sensitivity of other tissue cells is seen, in view of the widespread use of the skin reaction in assessing the sensitivity of the individual to tuberculin, and the possibly erroneous but nevertheless common practice of prognosticating the future progress of the disease from the degree of sensitivity thus demonstrated.

Chapter 3PRESENT INVESTIGATIONPLAN OF PRESENT INVESTIGATION

This investigation as envisaged consisted in the comparison, in patients under treatment in the sanatorium, of the local and the systemic response to tuberculin. It is clear that methods of tissue culture involving estimations of the specific cytotoxic index and measurements of the phagocytic activity of cells as used in animal experiment are unsuitable for the investigations of the cytotoxic action of tuberculin in man. Two of the most readily accessible of the body tissues, however, the skin and the peripheral blood, can be used and a technique was elaborated for the measurement of the responses of these tissues to tuberculin, the reaction in the skin being regarded as an indication of local response while that of the blood was regarded as an indication of systemic response.

With each patient the investigation was, therefore, divided into two parts:

- A. Intracutaneous tests with serial dilutions of tuberculin.
- B. A study of the changes which take place in the leucocytes of the blood when a sample is incubated with tuberculin at 37°C.

The plan of the investigation may be summarised for convenience as follows:-

- I. Observations on the Accuracy of Tuberculin Testing.

The/



The making of a special tuberculin syringe.

Technique used in this investigation.

## II. Observations on the Prevention of the Clotting of Blood and the use of Anticoagulants.

Experiment 1: To determine the minimum amount of heparin which would effectively prevent coagulation of blood during incubation without interfering with the staining properties of the leucocytes.

Experiment 2: To compare the anticoagulant powers and effect on the leucocytes of two preparations of heparin.

## III. The Action of Tuberculin on Leucocytes.

Experiment 3: Preliminary tests to ascertain the effect of the admixture of tuberculin with blood on the staining properties of the cells in (i) a sensitive individual as shown by positive tuberculin skin tests (ii) a non-sensitive individual as shown by negative tuberculin skin tests.

Also to ascertain the duration of interaction between blood and tuberculin to be allowed in the main investigation.

## IV. Main Investigation.

A. Object: (i) To investigate further the nature of the response to tuberculin of the leucocytes of a sensitised individual.

(ii) To compare this response with that of the skin to tuberculin in the same individual.

B. Further details of method.

C./

C. Analysis of data.

- (i) Changes in cell morphology.
- (ii) Changes in cell counts.
- (iii) Relationship to skin sensitivity.

I. Observations on the Accuracy of Tuberculin Testing.

It has been amply demonstrated through the years that the intracutaneous method of testing the skin sensitivity to tuberculin, as described by Mantoux in 1908, is the most reliable and accurate method as yet elaborated. That it is the only method which can be used with any degree of accuracy in serial or quantitative testing was concluded by P. D'Arcy Hart (1932) in the Medical Research Council publication "The Value of Tuberculin Tests in Man".

The making of a special tuberculin syringe.

In an investigation of the nature envisaged here where the accuracy to be achieved is necessarily extremely important, it was felt that the average tuberculin syringe presented several possibilities of error. The most serious of these is due to leakage at two points, viz:- at the junction between the needle and the nozzle of the syringe, and backwards past the piston, as the pressure required to introduce fluid between the layers of the skin is sometimes considerable. Consequently, an endeavour was made to find a syringe which would overcome these difficulties, the more troublesome being the former. A syringe, similar in pattern to the dental type syringe but fitted with graduated cartridges, was contemplated but, unfortunately, present day difficulties in manufacture rendered this impossible. With the co-operation of Messrs. Vicarey/

Vicarey and Davidson Ltd. (Glasgow), however, a syringe has been evolved which experience shows offers a high degree of accuracy.

The most important feature of this syringe is the screw type nozzle on to which the lead butt of the intradermal needle is firmly attached by means of a spanner. This ensures a completely watertight joint, obviating the leakage at this point. The accompanying photograph of the syringe and its component parts illustrates the simplicity of its design. The graduated plunger staff is useful, as the bevel of the needle cannot always be brought in line with the graduations on the barrel when it is screwed firmly into place. The stop on the plunger ensures that only the required amount of fluid will be injected even when the barrel markings cannot be seen.

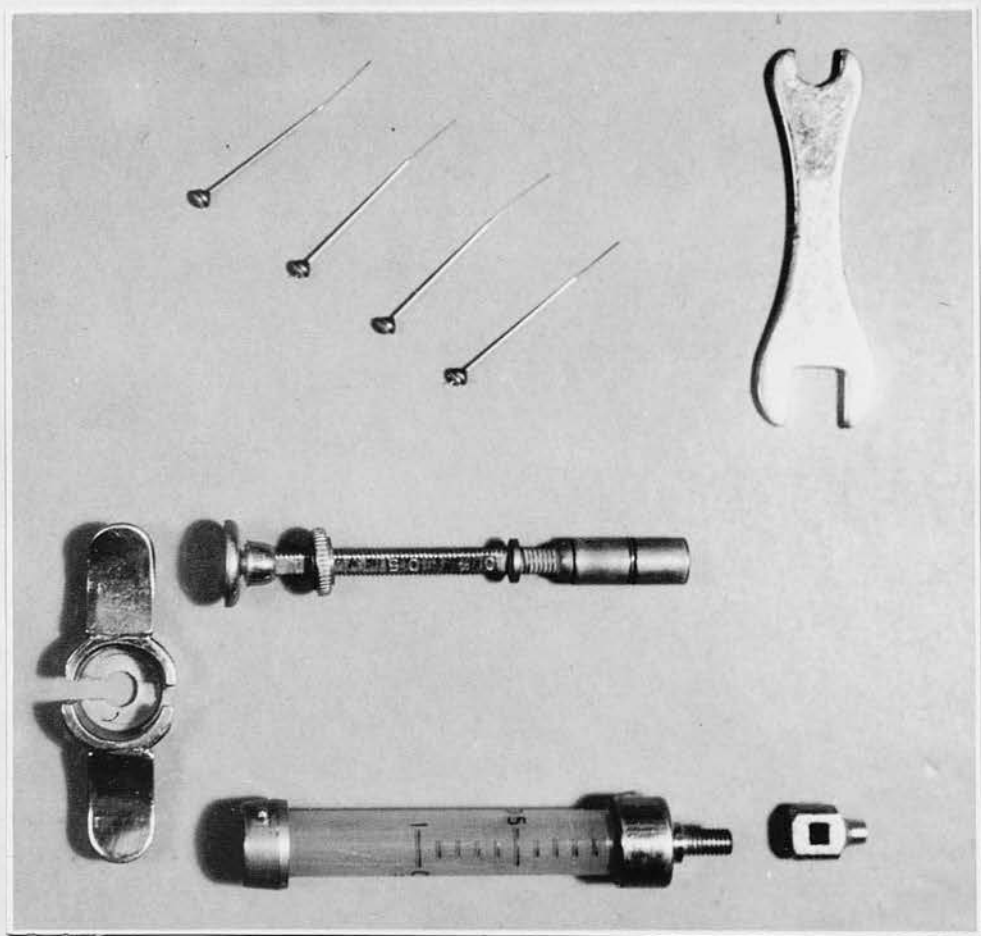


Fig. 1  
Tuberculin Syringe



### Technique of skin testing.

The technique of the skin testing in this investigation was in all other respects identical with that recommended by P. D'Arcy Hart, viz:

0.1 ml. of the serial dilutions of tuberculin was injected into the flexor surface of both fore-arms so that a well marked white bleb was raised.

The reactions were read 48 hours following injection and the following observations recorded:

Area of erythema - measurement of both horizontal and vertical diameters.

Inside area of pallor, if present, measured as above.

Induration of skin - measure of the increase in diameter of pinched skin and subcutaneous tissues as compared with that of a corresponding normal area.

The tuberculin used throughout the investigation was a preparation of "Old Tuberculin" obtained from the Royal College of Physicians Laboratory, Edinburgh. Serial dilutions 1:100 to 1:1,000,000 were made up at monthly intervals with full aseptic precautions using sterile normal saline as the diluent. The prepared solutions were stored at 4°C.



## II. The Prevention of Clotting of Blood and the Use of Anticoagulants.

The exact mechanism of the coagulation of the blood is not yet fully understood, and several theories are held, the most universally approved being the theory of Morawitz. According to this theory the mechanism can be summarised thus:  
 Prothrombin + calcium ions + thrombokinase = thrombin  
 Thrombin + fibrinogen = fibrin, the solid substance in the blood clot. Thrombokinase is liberated from the platelets when blood is shed and thrombin thus formed. Thrombin, by an action analogous to that of an enzyme, then causes the conversion of fibrinogen to fibrin.

A further theory, however, has been postulated by Howell who extracted a substance from liver which inhibits the coagulation of blood. This substance he called heparin. It is his belief that prothrombin and calcium ions alone will form thrombin and that heparin, by acting as an anti-prothrombin in the circulating blood, prevents this occurring. When the blood is shed, however, the thrombokinase liberated from the platelets neutralises the heparin and thrombin is then free to act with the fibrinogen to form fibrin.

The following methods may be used, therefore, to prevent clotting in blood:-

- (1) By drawing the blood into a container which is lined with a non-water-wettable substance such as paraffin/

paraffin wax. This prevents the disintegration of the platelets and will, therefore, delay the production of thrombokinase.

(2) Prevention of platelet disintegration by cooling the blood rapidly to 0°C.

(3) Removal of the free calcium ions by chemical substances which produce precipitation of an insoluble calcium salt. Substances commonly used for this purpose are sodium citrate, potassium oxalate, and a mixture of ammonium and potassium oxalate recommended by Wintrobe. Although ammonium oxalate causes the corpuscles to swell and potassium oxalate causes them to shrink, this latter mixture is so balanced as to prevent any alteration in the size of the cells as measured by the volume of packed cells.

(4) By the use of heparin which, as explained above, acts as an antiprothrombin. It has been found by comparison with haemophilic blood, for which no anticoagulant need be used, that heparin does not alter the size of the blood corpuscles.

(5) Hirudin, an extract made from the salivary glands of the leech, can also be used. It acts as an antithrombin and so prevents clotting.

In selecting an anticoagulant suitable for this investigation, the choice fell upon the biological substance heparin which, because of its nature and because of its proved properties, seemed least likely to damage the white blood corpuscles. It was of particular importance not to alter the staining/

staining properties or the morphology of the cell especially in view of the fact, that the changes, if any, that were likely to be manifest in the course of the investigation would possibly be very subtle.

It was also decided to use paraffin-waxed tubes, not so much for the part they would play in the prevention of clotting, but also because of the fact that the non-water-wettable surface would prevent, in part at least, adhesion of the cells to the tube and thus minimise mechanical damage to them. An attempt was made to secure plastic test tubes since plastic materials also present a non-water-wettable surface. Difficulties of supply, however, prevented delivery and the old-fashioned device of waxed glass was of necessity adopted.

#### Experiment 1

##### Object:

To determine the minimum amount of heparin which would effectively prevent coagulation of blood during incubation without interfering with the staining properties of the leucocytes.

##### Method:

Materials - HEPARIN B.D.H. of strength 500 international units/ml.

Paraffin-waxed test tubes

Whole blood

Normal saline

The preparation of heparin as above was diluted with sterile normal saline to give the following strengths:/

strengths:

- (i) 20 units/ml.
- (ii) 4 units/ml. (This strength as recommended for addition to blood for transfusion).
- (iii) 0.8 units/ml.

1.0 ml. of whole blood obtained by venepuncture was then added to 1.0 ml. of each of these solutions in a waxed tube. The tubes were incubated at 37°C for 24 hours. The tubes were examined for coagulation at intervals and films of the uncoagulated specimens spread on glass slides. These were stained by Leishman's method and examined under 1/12" oil immersion for changes in staining properties or morphology of the white blood corpuscles by comparing these slides with slides made of the same blood when fresh.

#### Results:

Coagulation: In solution (iii) containing 0.8 units heparin/ml. clotting of the blood took place after three hours' incubation.

In solution (ii) containing 4 units/ml. heparin very slight clotting began at the twelfth hour but had not advanced any in twenty-four hours.

No coagulation took place during the period of observation in solution (i) containing 20 units heparin/ml.

Staining properties: Little difference was noted in the staining properties of either the leucocytes or the erythrocytes during the course of the experiment.

Morphology of cells: Little change was noted in the appearance/



appearance of the polymorphnuclear cells or the lymphocytes up to the sixth hour but the monocytes had at this time undergone degeneration as evidenced by fragmentation and vacuolation of the cytoplasm and distortion of the nucleus. This change began to affect the polymorphnuclear cells at twelve hours and was extensive by the twenty-fourth hour. No great change was noted in the appearance of the lymphocytes at twelve hours. Degeneration, as evidenced by condensing of the nucleus, was noted at twenty-four hours, but this degeneration was not so marked as in the other varieties of leucocytes.

#### Conclusion:

The optimum dilution of heparin to satisfy the conditions set out in the object of the experiment is approximately twice the strength that is recommended for blood transfusion.

#### Experiment 2:

##### Object:

To compare the anticoagulant powers and the effect on leucocytes of two preparations of heparin -

HEPARIN (B.D.H.) 500 International units/ml.

LIQUEMIN (Roche) 1,000 Toronto units/ml.

(Recommended dilution for blood transfusion 2.5 Toronto units/ml.)

##### Method:

Materials - Solution of HEPARIN in normal saline of strength 100 International units/ml.  
Solution of LIQUEMIN in normal saline of strength/



strength 50 Toronto units/ml.

Whole blood

To two 2 ml. samples of blood 0.2 ml. of each of these solutions was added and the mixture incubated in paraffin-waxed tubes for twenty-four hours. Films made at intervals throughout the twenty-four hours were compared with films of fresh blood, special note being taken as before of changes in the appearance of the leucocytes.

Results and Conclusions:

- (1) The general impression obtained is that "Liquemin" in the strength used has less effect on the morphology of the cells than "Heparin".
- (2) Both substances have equal properties in the prevention of clotting.
- (3) "Liquemin" of strength 50 Toronto units/ml. should therefore be used as the anticoagulant in future experiments.
- (4) The smaller volume of heparin-saline solution used in this experiment permitted the more satisfactory spreading of blood films.

A selection of the slides were stained by the Jenner-Giemsa method in this experiment with more consistent staining of the leucocytes than in the films of the previous experiment stained by Leishman's method.

### III. The Action of Tuberculin on Leucocytes.

#### Preliminary tests.

#### Experiment 3:

#### Object:

To ascertain the effect of the admixture of tuberculin with blood on the staining properties of the cells in (i) a sensitive individual as shown by positive tuberculin skin tests: (ii) a non-sensitive individual as shown by negative tuberculin skin tests.

#### Method:

- (1) Intradermal tests were carried out on each subject with 0.1 ml. of dilutions of tuberculin 1:1,000; 1:10,000; 1:100,000; 1:1,000,000. The technique was as previously described.
- (2) At the same time a sample of venous blood was withdrawn into a syringe which had previously been washed through with the liquemin solution (50 T.U./ml.).
- (3) 1.0 ml. of this blood was added to a waxed tube containing
  - (i) 0.1 ml. liquemin solution (50 T.U./ml.).
  - (ii) 0.1 ml. tuberculin, dilution 1:100. The final dilution of tuberculin in the blood was therefore 1:1,000.
- (4) Similarly 1.0 ml. of the blood was added to a waxed tube containing
  - (i) 0.1 ml. liquemin solution (50 T.U./ml.).
  - (ii) 0.1 ml. normal saline.

These tubes were incubated at 37°C for twenty-four hours/

hours.

(5) Films of the blood were spread when fresh and of each specimen at the following times during twenty-four hours: 10 minutes: 20 minutes: 30 minutes: 1 hour: 2 hours: 3 hours: 6 hours: 9 hours: 12 hours: 24 hours.

These films were stained by the Jenner-Giemsa method, the technique of which was standard throughout the investigation and was as follows:-

(i) Slide flooded with eosin-methylene blue (Jenner's stain) and allowed to act for three minutes.

(ii) Equal quantity of buffered diluent\* added and allowed to act for one minute.

(iii) Slide drained and flooded with diluted Giemsa stain (8 drops stain to 5 ml. buffered diluent).

(iv) Slide washed, dried and mounted.

(6) The stained films of the tuberculin treated and the control samples of blood were examined under the 1/12" oil-immersion lens.

\* A stock solution is made by dissolving 25 gm.  $\text{Na}_2\text{HPO}_4$  and 32.5 gm.  $\text{KH}_2\text{PO}_4$  in 100 ml. distilled water. For use this is diluted to an approximately 2% solution (Whitby and Britton, 1946).

RESULTS:

Examination of the films made in this experiment for variations in staining properties of the leucocytes which might denote a toxic action of tuberculin on these cells resulted in the following findings:-

(1) The staining properties of the cells seemed little changed and comparison between the tuberculin treated blood and the control series in this respect showed little difference in either subject.

(2) Degeneration of the individual elements of the blood was evidenced as follows:-

(a) Neutrophil polymorphnuclear cells - distortion, disintegration and vacuolation of cytoplasm, loss of reticulation and partial condensation of nucleus.

(b) Lymphocytes - disintegration of cytoplasm with, in some cases, pseudopodial projections of cytoplasm. Loss of reticulation and condensation of nucleus.

(c) Monocytes - distortion, disintegration and marked vacuolation of cytoplasm. Disintegration and vacuolation of nucleus.

(d) Eosinophils - little noticeable change apart from distortion.

(e) Basophils - numbers too small for conclusions to be drawn.

These changes were present in all slides and affecting an increasing number of cells as the incubation/

cubation period lengthened. They appeared more quickly and were more extensive in the tuberculin treated series in subject (i). The monocytes were most rapidly affected, the lymphocytes showed degeneration only slowly, some appearing normal in the twenty-four hours slides, but their appearance was most easily altered by variations in the spreading and the staining of the films.

(3) Morphological changes attributable to the toxic action of tuberculin appeared limited to swelling of the cell as a whole with swelling and disappearance of the granules in the neutrophil cells of the polymorphnuclear group. This did not affect the cells to a measurable degree and was only apparent in the tuberculin treated series of subject (i). It was not a universal finding, in that all the cells seen did not show this change. This finding of swelling of the neutrophil series was most apparent in the film of the tuberculin treated blood of subject (i) made following twenty-four hours' incubation and the contrast in the size of the cells between this slide and the film of the control blood was appreciable.

(4) It was, however, noted during the examination of the films for the above changes that the leucocytes, and in particular the neutrophil polymorphs, of the tuberculin treated series in subject (i) were scarcer than in comparably spread films of the control series taken at the same interval.

It was therefore decided, in view of this fact and/



and also because the cells in all slides were so liable to distortion, particularly as the age of the specimens increased, to carry out a differential leucocyte count on all the films in the experiment. The results of these counts are given below in Tables 2 and 3 and the figures relative to the neutrophil group graphically represented in Figs. 2 and 3.

Results of the tuberculin skin tests, also given, (Table 1), showed that subject (i) possessed a high degree of skin sensitivity, while in subject (ii) no response to tuberculin was exhibited for any dilution.

TABLE 1/

T A B L E 1Experiment 3: Subject (i)

Results of intracutaneous tests (recorded in millimetres).

REACTION	DILUTION OF TUBERCULIN			
	1:1,000,000	1:100,000*	1:10,000	1:1,000
Area of erythema	17 x 17	15 x 15	25 x 25	44 x 60
Inside area	5 x 5	5 x 5	7 x 7	15 x 20
Vesiculation	-	-	-	-
Induration	5	3	9	14

\* Slight amount of leakage at site of injection.

Subject (ii)

Results of intracutaneous tests: No reaction obtained to dilutions 1:1,000,000; 1:100,000; 1:10,000 and 1:1,000.

T A B L E 2/

TABLE 2

Experiment 3: Subject (i)

Differential leucocyte counts (%) of the tuberculin treated and control samples of blood from subject (i).

Time Interval	Neutrophils		Lymphocytes		Monocytes		Eosinophils	
	Tuberculin %	Control %	Tuberculin %	Control %	Tuberculin %	Control %	Tuberculin %	Control %
Fresh	52.0	52.0	24.0	24.0	17.0	17.0	5.0	5.0
10 mins	51.0	54.5	30.0	37.0	7.0	6.5	1.0	1.5
20 mins	43.0	54.5	45.5	37.5	6.5	2.0	5.0	2.0
30 mins	46.0	54.0	47.0	40.0	4.5	6.5	1.5	0
1 hr.	38.0	66.0	54.5	31.0	4.5	2.5	4.0	0.5
2 hrs.	36.5	62.0	61.5	31.5	0.5	5.5	0.5	2.0
3 hrs.	37.5	58.5	55.5	37.0	4.0	2.0	2.0	2.5
6 hrs.	34.5	65.5	56.5	29.5	6.0	2.5	3.5	2.0
9 hrs.	33.5	76.5	58.0	19.5	3.0	3.0	4.0	0.5
12 hrs.	34.5		59.0		4.0		2.0	
24 hrs.	38.0	67.0	54.5	30.0	4.0	2.5	2.0	0.5

Note: Basophil percentages not included in Table as numbers are negligible.

TABLE 3/

TABLE 3

Experiment 3: Subject (ii)

Differential leucocyte counts (%) of tuberculin treated and control series of blood samples from subject (ii).

Time Interval	Neutrophils		Lymphocytes		Monocytes		Eosinophils	
	Tuberculin %	Control %	Tuberculin %	Control %	Tuberculin %	Control %	Tuberculin %	Control %
Fresh	63.0	63.0	24.5	24.5	4.5	4.5	8.0	8.0
10 mins	58.5	58.5	24.5	29.5	5.0	5.5	11.0	6.5
20 mins	51.0	51.5	23.5	23.0	10.0	6.5	10.5	9.0
30 mins	53.0	60.0	30.0	25.0	8.5	8.0	7.0	7.0
1 hr.	66.6	55.0	23.0	27.5	4.0	6.6	7.0	11.0
2 hrs.	55.5	52.0	30.0	28.5	6.5	8.0	6.5	10.0
3 hrs.	47.0	58.0	39.5	30.5	7.5	4.0	6.0	7.0
6 hrs.	50.5	57.0	35.0	30.0	1.5	4.0	12.0	9.0
9 hrs.	54.0	55.0	36.0	26.0	2.0	10.0	8.0	8.0
12 hrs.	54.0		27.0		9.0		7.0	
24 hrs.	43.0	39.0	45.0	47.0	4.0	5.0	8.0	9.0

FIG. 2/

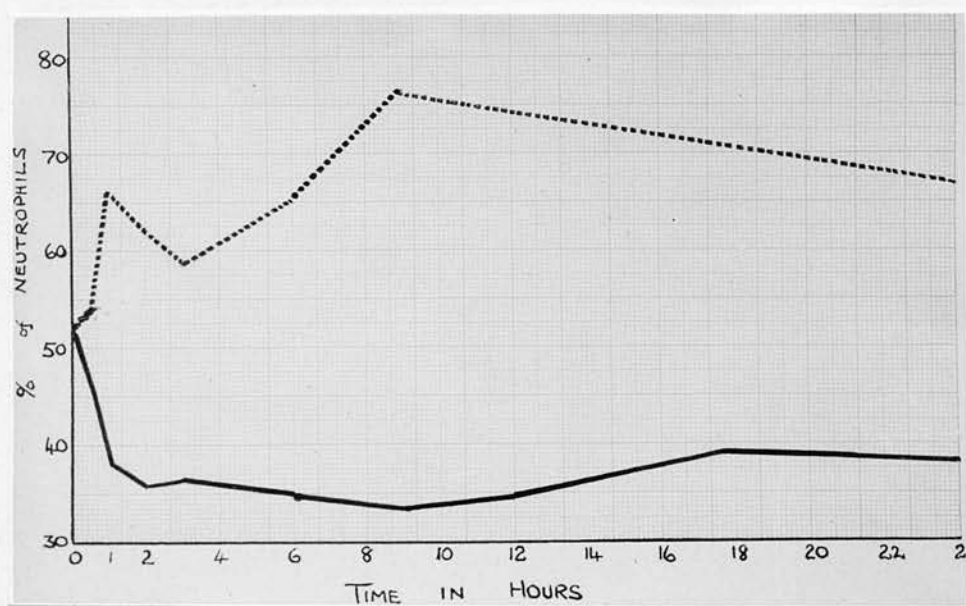


FIG. 2.

Percentage figures obtained for the neutrophil polymorph cells when samples of blood obtained from Subject (i) were incubated for 24 hours. The continuous line represents the tuberculin treated blood and the broken line the untreated control series.



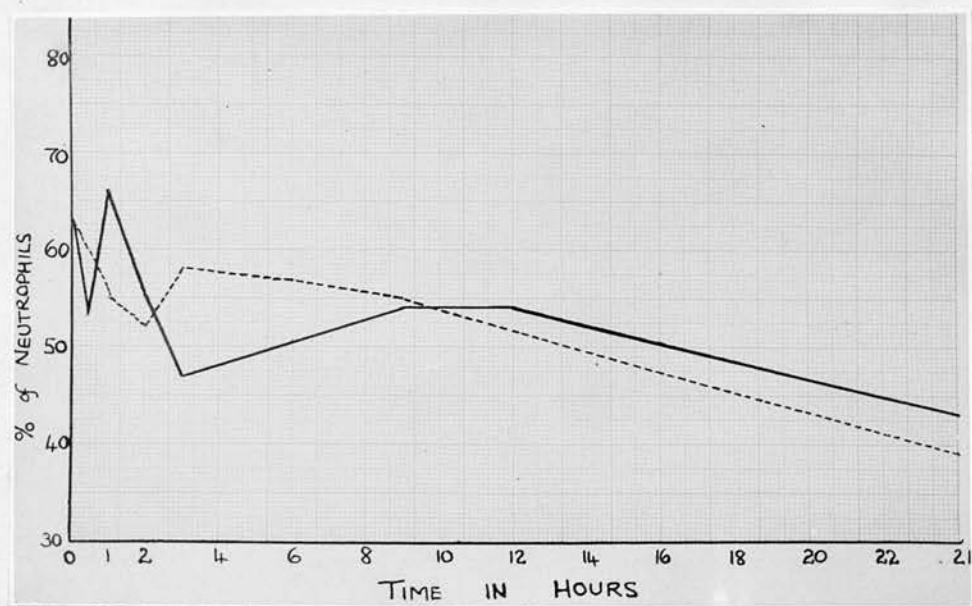


FIG. 3.

Percentage figures obtained for the neutrophil polymorph cells when samples of blood obtained from Subject (ii) were incubated for 24 hours. The continuous line represents the tuberculin treated blood and the broken line the untreated control series.

It is not proposed to comment in the text on every figure in these tabulated data. Nevertheless certain interesting facts should be noted. In the highly sensitive individual (Table 2) the films made after 1, 2 and 3 hours showed pronounced differences in their neutrophil polymorph content according to whether they had or had not been treated with tuberculin. Thus the treated blood contained only from 36.5%- 37.5% neutrophils whilst the untreated control blood of the same person contained 55.5%- 61.5% neutrophils. On the other hand in the non-sensitive individual (Table 3) there was no such change and after 1 and 2 hours' incubation there were actually more neutrophils in the treated blood than in the control blood. At 3 hours some diminution in the neutrophils had taken place. But broadly it seems that a scrutiny of the figures shows that the action of tuberculin on sensitive leucocytes manifests itself quickly and is appreciable at the end of one hour's incubation. Following this initial period, the degenerative process slows down and, although destruction of the cells takes place in the ensuing hours, it is to a considerably smaller degree.

### DISCUSSION

It is realised that no conclusion can be advanced from the results of one experiment with data on two cases and, in any case the whole question of experimental error is yet to be discussed (See page 64). Nevertheless it was decided, in the light of experience/

experience gained, that in the main investigation as envisaged observations on the cells could be confined to changes noted during three hours' incubation.

The reasons for this decision were as follows:-

(i) It was likely that in the majority of cases the action of tuberculin on the leucocytes would be similar to that described above and be well manifest by at least three hours. It would also be unlikely to advance appreciably further in a subsequent period.

(ii) By choosing a period of three hours it was probable that in all cases the action of tuberculin, if it were to be manifest, would be shown by this time even in an individual less highly sensitive than in the example cited.

(iii) Simple degeneration of the cells takes rather longer to be clearly demonstrated ( See Exp.1.).

(iv) Although it would have been ideal to have observed the changes over a period of twenty-four hours in each case, this was clearly impossible with the resources and time available and it was thought that by choosing three hours the investigation could be brought within manageable proportions while at the same time yielding information of possible value.

#### Preliminary Conclusions

(1) The leucocytes, in particular the neutrophil polymorphnuclear cells of an individual sensitised to tuberculin, as evidenced by positive tuberculin skin tests, are susceptible to the toxic action of tuberculin./

tuberculin. This sensitivity is illustrated by acceleration of the disintegration of the polymorphs in the presence of tuberculin.

(2) The leucocytes of a non-sensitive individual do not show the same susceptibility to the action of tuberculin.

#### IV. MAIN INVESTIGATION.

##### A. Object:

- (i) To investigate further the nature of the response to tuberculin of the leucocytes of a sensitised individual.
- (ii) To compare this response with that of the skin to tuberculin in the same individual.

##### B. Further details of method:

- (i) Intradermal tests with 0.1 ml. of serial dilutions of tuberculin were performed as before. Systemic reactions in properly performed Mantoux tests are rare, but in view of the possibility of severe local reactions in patients suffering from active tuberculosis the tests in the majority of cases were confined to the dilutions 1:1,000,000; 1:100,000; 1:10,000.
- (ii) Incubation of samples of heparinised blood with tuberculin as before using as a control a similar sample incubated with saline replacing the tuberculin. In this series of experiments 2.0 ml. of blood was added to 0.2 ml. of Liquemin solution and 0.2 ml. 1:100 tuberculin or 0.2 ml. normal saline resulting in, as before, a concentration of tuberculin in the blood of 1:1,000.

For the reasons noted above on page 38

it was decided that in this series the observations could be confined to the first three hours. Total white/



white blood cell counts with differential leucocyte counts were carried out on all specimens when fresh and at intervals up to three hours of incubation. In the nature of the technique chosen for this investigation, dilution of each sample of blood one-fifth of its volume with the mixture of anticoagulant and tuberculin in the one case, and anticoagulant and saline in the other took place. As, however, this dilution was constant in all cases and the experiment consisted in comparing one sample with another, it was not thought necessary to make an adjustment in the figures to allow for the dilution.

In view of the well recognised possibilities of error in making differential white counts, every effort was made to eliminate the mechanical error (See page 64) as far as possible. To achieve this slides were boiled for five to ten minutes in soap solution (2oz. soft soap to 1 pint water) and polished before use. Parallel lines were scratched on the back of the slide with a diamond pencil and the small drop of blood from which the film was made was placed at a constant distance from these lines. The film was spread at right angles to the lines and, in this way, as nearly as possible the part of the film used for the count was constant. Only evenly spread, thin films were used. 400 cells were counted when available but in the later films, when the number of cells had diminished, it was sometimes necessary to limit the number to 300 or 200. In these/

these cases, however, the field covered was extensive and this was thought to render the result reasonably accurate.

As regards the counting of the total number of leucocytes per cmm. in the blood, it was found that it was impossible to obtain two pipettes which gave identical results for the same specimen of blood. For this investigation, therefore, in which minor variations normally unimportant were to be avoided if possible, two pipettes which gave the closest approximation were chosen, the pipette used for each series, the tuberculin treated and the control, being kept constant.

#### Results:

Complete protocols embodying all experimental data from 14 patients (in one of whom two separate investigations at different stages in his disease were carried out), exhibiting varying degrees of hypersensitivity to tuberculin are available for consultation in the appendix to this work. Since over 100 differential counts with the calculation of absolute numbers in each case are involved, all the facts could not appropriately be presented here.

C. ANALYSIS OF DATA

Two types of result require scrutiny: firstly those based on changes in cell morphology, and secondly those based on cell numbers. The two are related inasmuch as any diminution in the numbers of certain cells following a possible necrotising action of tuberculin will be preceded by degenerative appearances in cell morphology. In practice, however, only the former can be accurately stated and changes in morphology are difficult to measure. Certain features require comment, nevertheless, even though at this stage in a tentative manner.

(i) Observations on changes in cellular morphology following the action of tuberculin.

(1) When the samples were incubated for three hours only, there was on the whole less differentiation between the cells of the tuberculin treated films and those of the control series as regards appearance and morphology. Nevertheless, although the changes were not of a measurable degree, it was apparent in most cases that the cells, and in particular the neutrophils, of the control series were in a less degenerate state than those of the tuberculin treated blood.

(2) As regards the changes in the granules of the neutrophil polymorphs, it would appear from a study of the slides that the first change which took place was an increase in the size/

size of the granule denoting some swelling. This was followed by the disappearance of the granules but in most cases this particular change had not reached this stage by the end of the third hour and, therefore, distinguishing of the treated and control films by this finding alone was impossible. Also it appeared that the cells from different subjects differed in the degree of granularity, but whether this was due to some slight variation in the technique it was difficult to say. Vacuolation of the cytoplasm which can be taken as an indication of degeneration, although present also in the control series, was seen to a greater degree in the tuberculin treated blood films.

(3) The impression was also gained that the young neutrophil polymorphs, as evidenced by lack of differentiation of the nucleus, were more quickly destroyed than the older cell with the well differentiated nucleus. The two types of cell also exhibited in some cases a slightly different reaction in that, whereas the nucleus of the older cell became condensed with loss of reticulation, the nucleus of the younger cell appeared to swell and become fragmented. This change was not confined to the tuberculin series as it was also present in the control slides but the same trend was noted here as with the other evidences of degeneration in the cells, namely that it appeared more quickly and was more complete in the tuberculin treated series.

(4)/



(4) An interesting illustration of the action of tuberculin on the young polymorph was shown in Subject 5 (b) where a considerable number of the neutrophil cells were tending towards the premyelocyte type with faintly basophilic cytoplasm and a very poorly differentiated nucleus. These cells, as the culture period lengthened, gradually became more basophilic and the nucleus assumed a "splintered" appearance. After three hours' incubation with tuberculin they had almost completely disappeared from the slide, while in the control film they were still comparatively frequently seen.

(5) It is considered that errors of interpretation are most likely to concern the monocyte percentage as a study of the films would appear to indicate a fairly wide variation in morphology in this series of blood samples. The well differentiated cell of classical description was seen comparatively infrequently. This may be due to the fact that many of the subjects were suffering from progressive tuberculosis in which the monocyte plays a large part and thus immature forms are more likely to be encountered in the peripheral blood. The rapid degeneration of the cell on incubation in vitro also added to the difficulties of interpretation, and it was again impossible in this further series to distinguish any certain change attributable only to the action of tuberculin. The signs of degeneration were, as before, vacuolation and disintegration of the/



the cytoplasm and the nucleus.

(6) It was noted that in certain slides the cells had a greater tendency to clump than in others and that the monocytes and the polymorphs were most liable to be affected in this way.

The mathematical analysis of such subtle changes as have been described is, for technical reasons, beyond the scope of this work.

(ii) Observations on changes in the leucocyte counts following the action of tuberculin.

Mention was made above that a diminution in the numbers of certain cells occurred following the interaction of blood and tuberculin. This statement must now be examined more closely, since it is based on the principal evidence obtained in this inquiry.

(1) The essential facts are presented in Table 4. In this table the results are classified as showing the fall in the individual blood leucocytes after three hours' incubation in fifteen different blood samples, two of these being taken from the same patient but in different stages of his disease. The control series, it will be recalled, were incubated, in place of tuberculin, with a bulk of saline equal to that of the tuberculin.

LEGEND/

LEGEND FOR TABLE 4

Fall in the individual blood leucocytes of  
fifteen different blood samples after three hours'  
incubation, one specimen from each being treated  
with tuberculin and another untreated.

T A B L E 4

Subject No.	Neutrophils		FALL IN ABSOLUTE NUMBERS				Eosinophils	
	Tuber- culin	Con- trol	Tuber- culin	Con- trol	Tuber- culin	Con- trol	Tuber- culin	Con- trol
1	1775	976	400	756	300	16	40	36
2	998	170	368	+340	312	306	16	0
3	3440	4690	260	110	1180	1234	20	158
4	2840	1222	+791	+882	331	190	77	77
5(a)	4177	253	80	+120	776	1237	204	126
5(b)	4422	4106	863	1268	32	156	58	126
6	3178	2178	+854	+1572	340	144	2	8
7	3599	3084	+1254	+1548	419	247	5	111
8	673	+222	482	74	157	196	87	142
9	2849	2949	+840	+1215	169	+130	10	88
10	1231	2056	+300	+ 140	138	16	132	30
11	2590	455	+1839	717	419	158	331	70
12	766	+3	+370	+24	271	325	33	1
13	3618	1962	620	+1660	140	93	+8	93
14	947	+691	+558	+604	95	92	107	193
MEAN	2474	1607	206	195	358.6	300.6	95	84
MEDIAN	2800	1200	+300	+150				
MODE*	3452	386	+618	+585				

\* taken as three times the distance from the mean that the median is, a method of calculating the mode described by Harvey and Hamilton (1934).

In the table it will be noted that wide variations occurred in the degree to which the neutrophils disappeared in 3 hours both in the treated and the control series. But a scrutiny of the individual cases shows that the trend was, with only three exceptions, more noticeable in the tuberculin treated bloods than in the controls. Indeed as will be observed from the foot of the table whichever method of recording the arithmetical average is adopted the disintegration of the polymorphs is more pronounced in the treated than in the untreated slides.

Graphically these figures are illustrated in an "ogive" or "array order" graph (Figure 4) where the ordinate represents the disappearance of polymorphs in absolute numbers from the blood samples in every case. The continuous curve represents the treated samples and the broken curve the control samples. Wide variations have clearly occurred but the important feature is the divergence of the medians in the two groups. Had there been no difference between the two groups (as in the case of the lymphocytes to be shown hereafter) the two medians would have nearly approximated. But where, as here, the medians have diverged widely some measure of reliability may be placed on the inference that the tuberculin has accelerated the disintegration of the polymorphs.

A similar graph has been prepared for the lymphocyte component of the leucocytes but here no accelerated/

accelerated destruction appears to have taken place as a result of the action of tuberculin. ( Fig. 5)

FIG. 4./



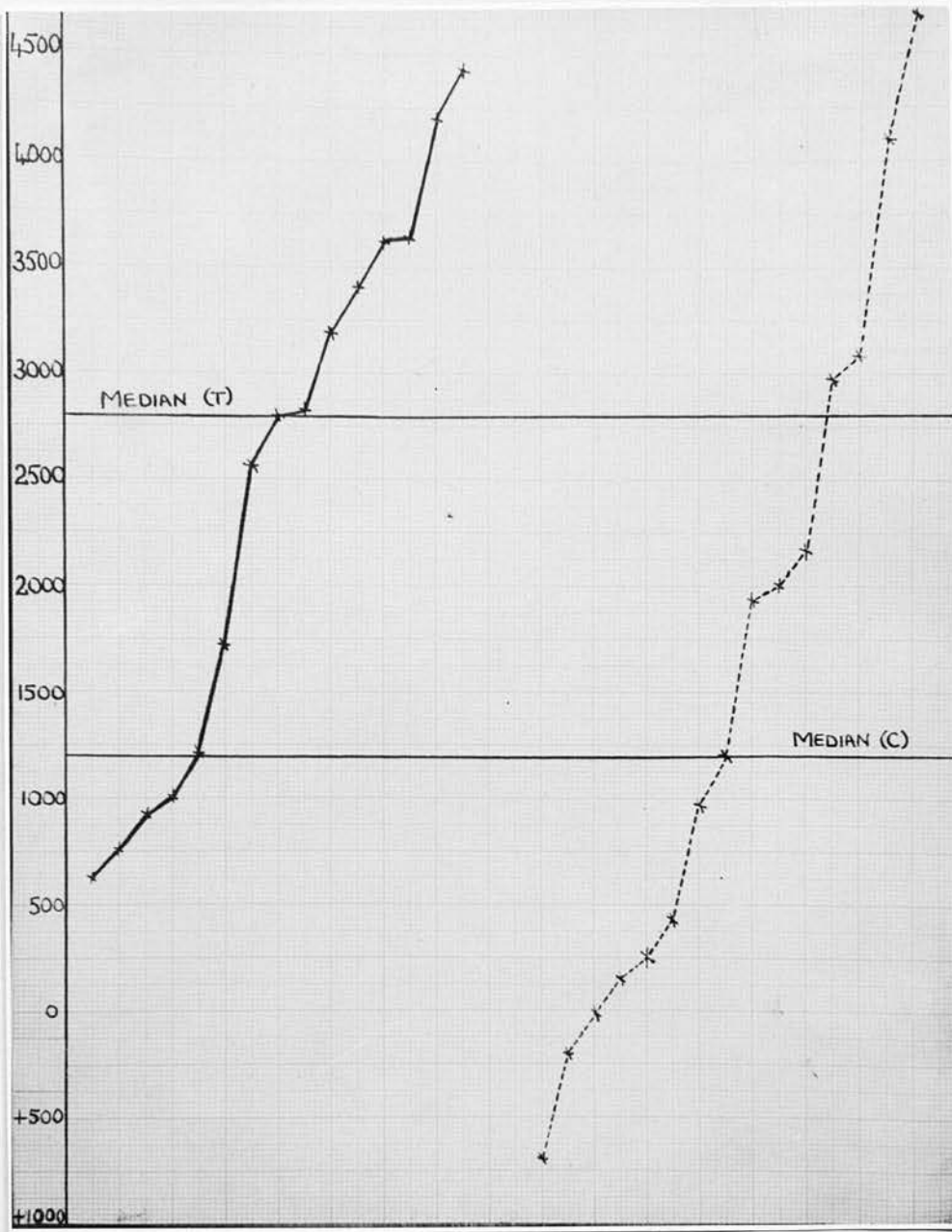


FIG. 4.

Ogive or Array order graph illustrating the disappearance of neutrophils in vitro from 15 samples of blood treated with tuberculin (T) and from 15 control samples (C). Tuberculin treated blood represented by continuous line; untreated control blood by broken line.



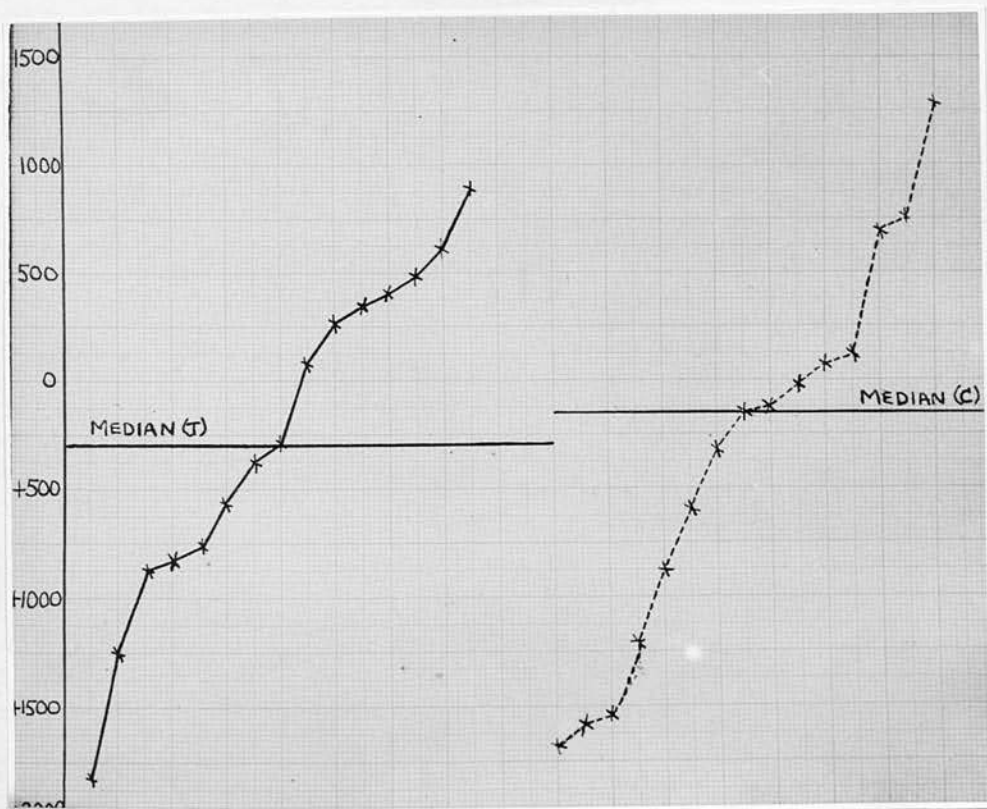


FIG. 5.

Ogive or Array order graph illustrating the disappearance of lymphocytes in vitro from 15 samples of blood treated with tuberculin (T) and from 15 control samples (C). Tuberculin treated blood represented by continuous line, untreated control blood by broken line.

The numbers of the other constituents of the white blood corpuscles are too small for any conclusion to be drawn, although in both the monocytes and the eosinophils a slightly accelerated rate of fall has occurred in the tuberculin treated bloods as compared with the control.

(2) A further method of analysing the data of the rate of disappearance of the neutrophil polymorphnuclear cells is shown in Table 5 where the percentage drop in the polymorphs has been calculated for the tuberculin treated series and the control series when the incubation period was three hours.

T A B L E 5/

TABLE 5

Percentage drop in neutrophil polymorphs calculated for the tuberculin treated and the untreated control blood after three hours' incubation.

Subject No.	% Drop in Neutrophils Tuberculin treated (T)	% Drop in Neutrophils Control Series (C)	T/C *
1	63.4	34.7	1.8
2	33.0	5.6	5.9
3	27.7	38.1	0.6
4	55.6	26.9	2.1
5(a)	61.5	36.6	1.7
5(b)	32.3	27.0	1.2
6	53.3	46.3	1.2
7	52.6	46.2	1.1
8	40.4	+ 15.8	+ $\infty$
9	36.2	27.3	1.3
10	24.7	37.2	0.7
11	57.0	10.0	5.7
12	16.2	0	16.2
13	47.7	18.7	2.5
14	12.3	+ 8.3	+ $\infty$

\* The figures in this column are regarded as "index figures" recording, in a comparative manner, the speed with which the polymorphs disappear in the tuberculin treated and the control series of blood because it is argued,

(i) if the polymorphs disappeared with equal speed in both tubes then this figure would be unity:

(ii) if the polymorphs disappeared more slowly in the tuberculin treated series, then the figure is below unity:

(iii) if the polymorphs disappeared more rapidly in the tuberculin treated series, then the figure is greater than unity.

Thus, in thirteen out of fifteen cases the polymorphs disappeared more rapidly in the tuberculin treated blood than in the control specimens.

(3) A scrutiny of the results (figures given in the appendix) shows than an appreciable fall in the total leucocyte count together with an appreciable fall in the percentage of neutrophil polymorphs, as compared with the corresponding figure for the control series, occurs in seven cases (subjects 1, 2, 4, 5a, 8, 11 and 12) and that in six of these seven the initial or fresh total count was below 10,000/cmm. Conversely, in the eight remaining cases (subjects 3, 5b, 6, 7, 9, 10, 13 and 14) where the falls in the tuberculin treated series were not appreciably greater than those in the control tubes, five of the eight exhibited initial total counts of 10,000/cmm. and over. This may mean that the figures/



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figures obtained by the usual methods of total and differential leucocyte counting are more accurate when the figure for the total count is not excessively high. It was found, for instance, that in performing differential counts where the cells were plentiful there was a greater tendency for aggregations of polymorphs and monocytes to be encountered. This would tend to increase the error and may account for an occasional discrepancy in the polymorph or lymphocyte percentage where a high monocyte or eosinophil percentage has been recorded.

It was also noted that in three of the cases in the second group above (subjects 3, 9 and 14) the fall in the total count and the fall in the neutrophil percentage was appreciable after one hour's incubation with tuberculin as compared with the corresponding figures for the control. Nevertheless, by the third hour, despite a further though less marked drop in the total count, the percentage of neutrophils had risen again, suggesting that the rate of disappearance of the lymphocytes, although slower initially, was accelerating.

(4) An attempt was made to estimate the significance of the drop in the percentage of polymorphs in the treated series as compared with the control by calculating the standard error of difference for each by means of the formula:-

$$e = p_0 q_0 \left( \frac{1}{n_1} + \frac{1}{n_2} \right) /$$

$$e = \sqrt{p_o q_o \left( \frac{1}{n_1} + \frac{1}{n_2} \right)} \quad (e^2 = p_o q_o \left( \frac{1}{n_1} + \frac{1}{n_2} \right))$$

where  $p_o = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}$

$n_1$  and  $n_2$  = number of cells counted in the two counts.

$p_1$  and  $p_2$  = proportion of polymorphs in the two counts.

If the observed difference is less than three times the standard error of difference, it may entirely be the result of chance. ( Barnett 1933)

The results are represented in Table 6.

#### TABLE 6/

TABLE 6

Table showing the calculated Standard Error of Difference (e) and the Observed Difference between the initial and final proportions of neutrophil polymorphs in the tuberculin treated and the control series of bloods after 3 hours incubation.

Subject No.	TUBERCULIN SERIES		CONTROL SERIES	
	e	Observed Difference	e	Observed Difference
1	5.0	15.0	* -	+1.0
2	4.0	4.0	3.7	1.0
3	3.3	3.0	3.3	2.5
4	5.0	22.5	4.8	13.0
5(a)	5.0	18.5	5.0	6.5
5(b)	* -	+2.0	* -	+6.0
6	4.8	21.0	4.9	29.5
7	5.0	35.5	5.0	35.0
8	5.8	5.0	* -	+7.0
9	3.6	15.0	3.6	17.0
10	3.7	6.5	3.7	12.5
11	4.4	25.0	* -	+3.5
12	3.4	4.5	* -	+3.5
13	* -	0	3.7	8.0
14	3.5	2.5	* -	+1.5

\* Calculation of the Standard Error of Difference was impossible owing to an increase in the percentage.

Falls in the proportion of tuberculin treated polymorphs as compared with the control series has therefore, occurred to a statistically significant degree in four cases. (Subjects 1, 4, 5(a), and 11.)

(iii) Relationship of the response to tuberculin of the skin to the response of the leucocytes of the blood.

For ease of reference and simplicity in presenting results, the degree of skin sensitivity shown by the patients was grouped as follows:-

An area of erythema 10 mm. or more in diameter with or without measurable induration was regarded as a positive result and taken as the basis for the system of grouping. The highest dilution of tuberculin for which a positive result was obtained was regarded as the threshold and the patient placed in the corresponding group. Each dilution includes two groups, the degree of the response determining the particular group in which the patient was placed.

T A B L E 7

Groupings of skin response to tuberculin.

Group	Dilution of tuberculin	Area of erythema (millimetres)
1	1:100	10 x 10
2	1:100	< 20 x 20
3	1:1,000	10 x 10
4	1:1,000	< 20 x 20
5	1:10,000	10 x 10
6	1:10,000	< 20 x 20
7	1:100,000	10 x 10
8	1:100,000	< 20 x 20
9	1:1,000,000	10 x 10
10	1:1,000,000	< 20 x 20



If within the limit of one dilution, induration more than 5 mm. in extent was manifest, the patient was moved up one group.

Thus, the higher the group, the higher is the degree of skin sensitivity to tuberculin.

As has already been observed an accelerated destruction of the neutrophil group of polymorphs as a result of the action of tuberculin has apparently been demonstrated. (See Tables 4 and 5). A similar action of tuberculin on the lymphocytes has not been demonstrated and the numbers of the other components of the leucocyte group are too small for conclusions to be drawn. Thus for the purposes of investigating the relationship of the response to tuberculin of the skin and the leucocytes the figures for the neutrophil group will be taken.

In order, then, to examine the degree of correlation between the amount of skin sensitivity demonstrated and the action of tuberculin on the neutrophil polymorphs as evidenced by the accelerated disappearance of these cells when incubated with tuberculin a scatter diagram (Fig. 6) has been prepared. The percentage drop in the neutrophils of the tuberculin treated blood (See Table 5) and the corresponding skin sensitivity group are plotted for 15 observations (14 patients). A similar graph (Fig. 7) was prepared where the action of tuberculin on the neutrophils is represented by the comparative index (T/C) calculated as explained on Page 55 (See Table/

Table 5). Again these figures for 15 observations (14 patients) are plotted with the corresponding skin sensitivity group.

Examination of these graphs would seem to show that there is no relationship between the skin sensitivity on the one hand and the sensitivity of the neutrophils represented either as the percentage drop after incubation with tuberculin or as the comparative index on the other.

Application of the  $\chi^2$  test to the four quadrants of the graphs results in a figure for p of 0.6 - 0.7. This would signify that 6 - 7 times in 10 similar figures could be obtained merely as a result of chance. Therefore it can be taken that no relationship has been demonstrated between the two types of tissue response under the conditions of the present investigation.

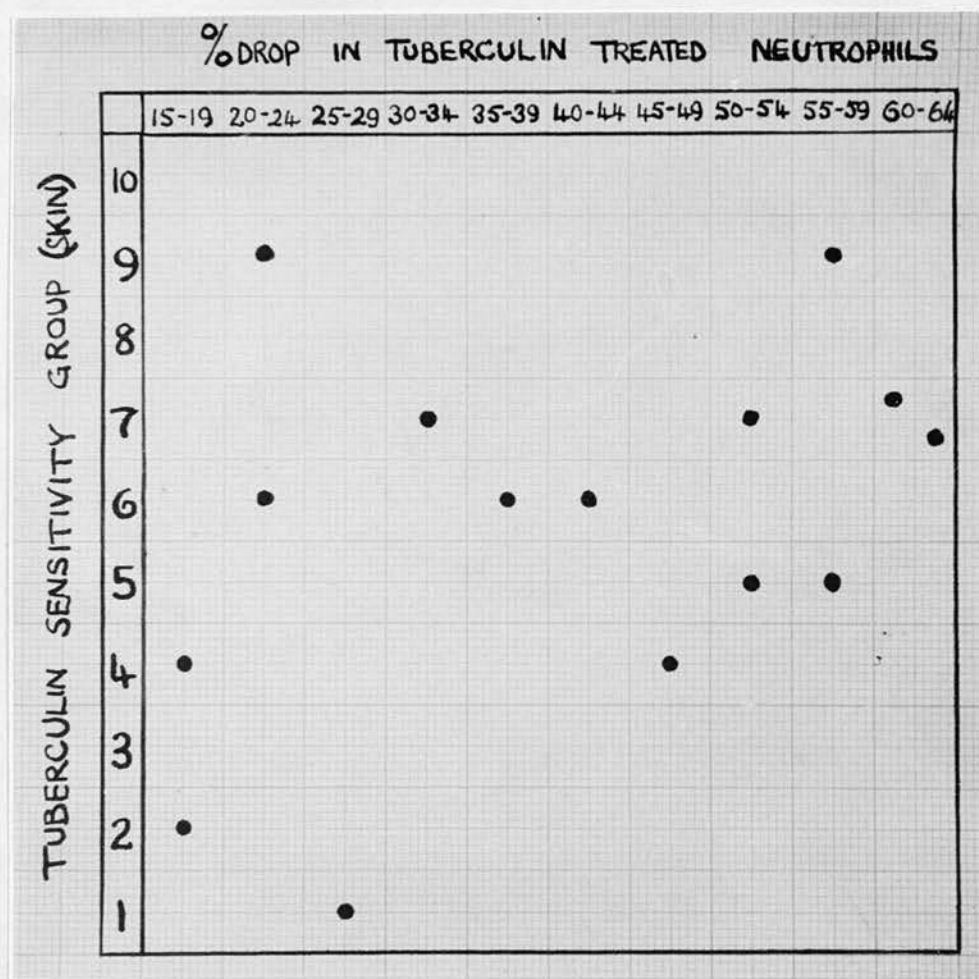


FIG. 6.

Scatter diagram to investigate the relationship between the disintegration of the neutrophil polymorphs in the presence of tuberculin and the tuberculin skin sensitivity (15 observations : 14 patients).

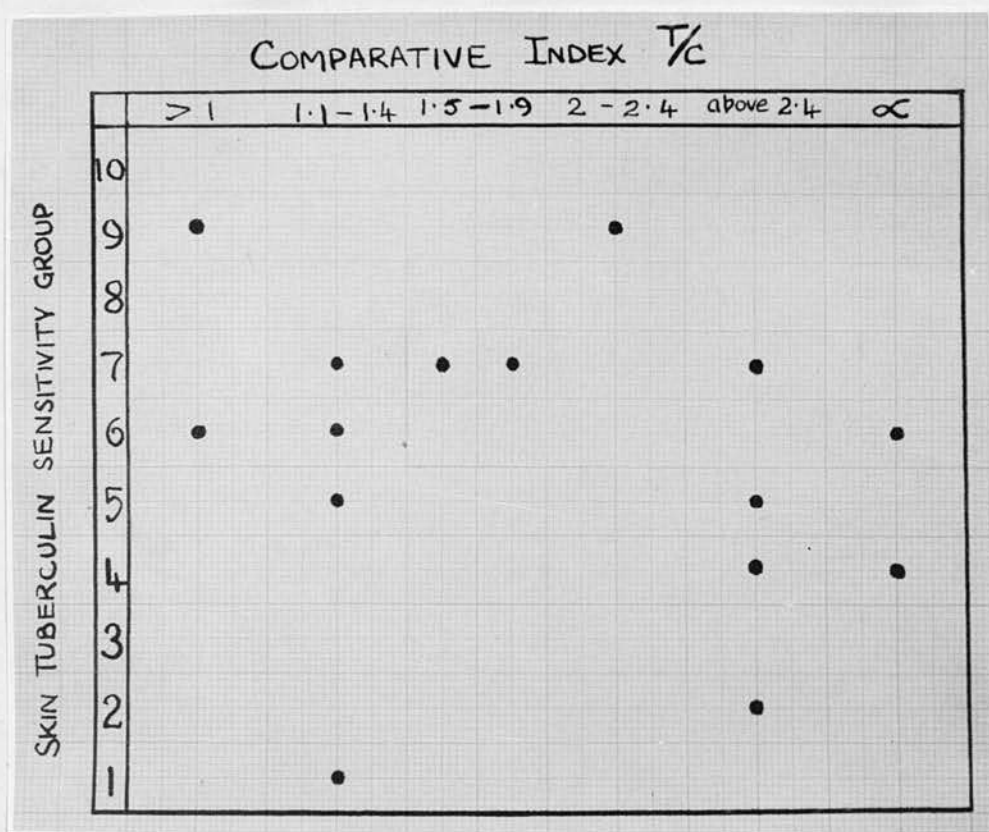


FIG. 7.

Scatter diagram to investigate the relationship between the disintegration of the neutrophil polymorphs (based on a comparative index) in the presence of tuberculin and the tuberculin skin sensitivity (15 observations : 14 patients).

For explanation of comparative index figure see text.

Chapter 4DISCUSSIONConditions of the experiment

Cell counts That the counting of the numbers of leucocytes in the peripheral blood is fraught with possibilities of error is well recognised and a considerable volume of literature has been amassed on the work of those who have investigated this problem. Barnett (1933) has studied the matter exhaustively and approaches it from the viewpoint of the statistician. He divides the possibilities of error into three categories:-

- (a) mechanical error
- (b) errors of interpretation
- (c) errors of chance.

Mechanical error

As regards the first of these, in this investigation every effort was made, as has already been explained, to eliminate it both in the counting of the total number of leucocytes in a cubic millimetre of blood and in the differential count of the blood film by the standardisation of the technique throughout the investigation. Nevertheless, it is impossible to eliminate it completely without artificial means to allow the spreading of identical films each time. It is clear also that the rise or fall in the percentage of any one component of the count of necessity means a corresponding reciprocal change in the percentage of the others. In an investigation of this kind, therefore, an unusually high percentage in one/



one film, possibly due to a chance distribution of cells, of any one type will produce a similar error in the opposite direction of the other components. We have noted the occurrence of this already in one or two of the films where an unusually high monocyte or eosinophil percentage caused what appeared to be a discrepancy in the neutrophil or lymphocyte percentage.

### Errors of interpretation

These are considered to be reduced to a minimum as all counts were made by the same observer. Despite this, however, it must be remembered that slight variations may occur even with the most practised worker when a second count is made on the same slide. Added to this, too, is the fact which soon becomes clear in the study of normal and abnormal blood cytology that the truly classical picture, as described, is not by any means always manifest even within the normal range. Thus, when degenerative changes are appearing interpretation becomes increasingly difficult. The cells which, in this series, presented the greatest problems were the monocytes and the large lymphocytes, particularly when atypical examples were encountered having, in the later stages, sometimes lost their characteristic distinguishing features. Consequently, it can be seen that a certain error may have arisen, but it is thought to be of less consequence in this investigation than the other types.

Errors/

### Errors of chance

It is clear that in the examination of the cells of the blood by means of the spread film we have available only a very small sample of the total number of cells in the blood as a whole. (Even in this investigation, where we were concerned with the fate of the cells in only 2 ml. of blood, the number of cells in one film represents only a very small fraction of the whole.) The error, therefore, which is inherent in the standard methods of blood examination, due to the fact of random sampling, has received the careful attention of many workers. Those who have applied a statistical approach to the problem have all arrived at a similar conclusion, namely that this error can under certain conditions be considerable but that it is reduced by increasing the number of cells counted (Barnett 1933, Plum 1936, Goldner and Mann, 1938). The counting of at least 400 cells is recommended and with this number Barnett reckons the error attributable to chance at  $\pm 7.5\%$  when the percentage of the total cells is in the 40% - 60% range. Stobie and England (1942) maintain, however, that the counting of 100 cells in a constant manner is as accurate as counting 200 cells. A statistical foundation for this statement does not accompany the work.

### Concentration of tuberculin

Workers, to whom reference has already been made, who have used the tissue culture method of studying/

studying the cytotoxic action of tuberculin have used varying concentrations of this substance. The results obtained, and in particular those of Aronson (1931), appear to show a quantitative action varying with the dilution of tuberculin used and non-specific results are obtained when the undiluted product is utilised.

In this investigation a concentration in the blood sample of 1/1,000 tuberculin was used but it is possible that with a greater concentration the toxic effect on the cells would have been more dramatically demonstrated.

#### Time factor

Similarly, prolongation of the incubation period would have increased the action of the tuberculin on the susceptible cells, but at the same time non-specific degeneration of the cells would have played an increasingly large part.

#### Results of Present Investigation

##### Cytotoxic Action of Tuberculin

The toxic action of tuberculin on the leucocytes of a sensitised individual would appear to be undisputed but the unequivocal demonstration of this fact in an investigation of the kind undertaken here is most difficult.

The action of tuberculin is that of a toxic substance in that it causes death of the cell, but under the conditions of the present experiment it is little more than an acceleration of the normal process/

process of degeneration which will take place when the cell is removed from its normal environment and source of nourishment. The neutrophils show swelling and eventual disappearance of the granules with disintegration and condensation of the nucleus.

Monocytes are particularly fragile and at an early stage show disintegration and vacuolation of the nucleus. The lymphocytes, possibly by virtue of their physical structure, show least in the way of toxic changes, but those, when present, consist in condensation of the nucleus and the development of amoeboid processes of the cytoplasm.

As explained above, these changes were not manifest to a measurable degree and recourse was had to the analysis of the figures obtained for the individual cell groups. In the chapter on analysis of data an attempt was made to make a statistical approach to the problem of interpretation of the figures (See Table 4).

It is considered that these figures represent an accelerated destruction of the leucocytes in the presence of tuberculin, this being manifest most strikingly in the neutrophil group of polymorphnuclear cells.

Exploring the figures further in an attempt to find a convenient formula to express the apparent destruction of the polymorphs, index figures representing the comparative drop in the tuberculin treated and the control series were worked out (See Table/



Table 5). These figures as already explained, demonstrated an acceleration in the tuberculin treated series in thirteen out of fifteen of the cases.

The employment of differential leucocyte counting in an investigation such as this imposes, if the criteria of Barnett are used, stringent statistical standards. Consequently, as represented in Table 6, drops in the polymorph percentage of the tuberculin treated bloods as compared with the control group were present to a statistically significant degree in only four cases.

Despite this, however, it would seem justified to assume that the toxic and necrotising action of tuberculin on sensitised cells has been demonstrated; and that, of the leucocyte series, the neutrophil group is the most susceptible. It is realised that the monocytes are also extremely susceptible but the numbers in this group are too small for any conclusion to be drawn.

#### Historical review:

It is interesting to note that the necrotising power of tuberculin on leucocytes was first recognised by Koch. An important series of experiments, clearly inspired by a study of Koch's work, was carried out by Holst (1922) and illustrated the effect of tuberculin on two of the well known properties of leucocytes, viz. migration and phagocytosis. These experiments were carried out in vitro on whole blood and inhibition of both of these properties/



properties took place in the presence of tuberculin. The morphological degeneration of the cells was also noted by this worker but a specific action on the sensitised cells of a sensitive individual was not demonstrated, possibly because of the use of a preparation of pure tuberculin.

#### Specificity of action of tuberculin:

Although this investigation is not concerned primarily with contrasting the toxic action of tuberculin on the cells of a sensitive individual and its non-toxicity to the cells of a non-tuberculous individual, it is appropriate to mention here that the specificity of the action of tuberculin has been demonstrated by many workers. Indeed, it is a characteristic of the tuberculin type reaction that the cells of the sensitised individual are sensitive in vitro to the sensitising antigen.

Although no conclusion can be drawn from the results of a single experiment, it can be assumed that the result obtained in Preliminary Experiment No. 3 is yet another demonstration of the specificity of the action of tuberculin. It will be remembered that in this experiment the action of tuberculin on the cells of a highly sensitive individual was contrasted with its action on the cells of a non-sensitive individual and, whereas the toxicity of tuberculin was clearly demonstrated in the former, little or no change beyond that of normal degeneration was noted in the latter.

Relative/

Relative action of tuberculin on polymorphs and lymphocytes:

In accordance with the fact mentioned above that the lymphocytes show least in the way of toxic changes we find, too, that the rate of disappearance of the lymphocytes is less than that of the treated neutrophil polymorphs. This apparent greater sensitivity of the polymorphs to tuberculin has also been demonstrated in tissue culture studies by Rich and Lewis (1932), and Heilman and Feldman (1944), but whether this difference in response is due to their differing physical structure or a manifestation of their physiological or functional properties it is beyond the scope of this enquiry to determine.

Local skin response and cytotoxic response compared:

The multiplicity of investigations to which reference has already been made in Chapter 2, gives some conception of the effort which has been made in the attempt to elucidate the response of the tissues to tuberculin. The countless numbers of enquiries into the skin tuberculin response since its inception is also evidence of the keen interest this aspect of sensitivity has aroused in the minds of tuberculosis workers everywhere. Comparatively little work has been done, however, to contrast or compare the two responses in the same subject.

Further reference can be made here to the interesting findings of Heilman and Feldman (1946) during their extensive series of tissue culture experiments/

experiments in tuberculous rabbits. It will be remembered that in animals whose tuberculin skin reaction was negative, either because the animals were in a moribund state or during the course of inter-current infection, the tissue cells in tissue culture still demonstrated sensitivity to the toxic effect of tuberculin. The number of animals in this series was admittedly small, but the view expressed by the authors is of considerable interest. It is as follows:- "The results of this present study affirm the view generally held at the present time that a spontaneous loss of cutaneous sensitivity does not necessarily indicate a loss or diminution of systemic hypersensitivity." They indicated the need for further work to elucidate the position in other states of "anergy" or loss of skin sensitivity.

A recent investigation (Kirchheimer and Weiser, 1948) reports the results obtained in tissue cultures from animals in whom complete loss of skin sensitivity had been obtained by desensitisation with tuberculin. In this series considerable diminution occurred in the sensitivity of the cells to the toxic action of tuberculin, but complete desensitisation of the tissue cells was not demonstrated as it was in the case of the skin.

That oestrogen reduces the inflammatory response of the skin to tuberculin in sensitised rabbits is a finding recently described by a group of American workers (Lurie et al, 1949) as a result of/

of investigation into constitutional factors in resistance to infection. These workers demonstrated the lack of correspondence between the response of the skin and of the blood leucocytes to tuberculin in oestrogen treated animals. It appeared that oestrogen reduced the local inflammatory response of the skin to tuberculin in sensitised rabbits by virtue of depressing the inflammatory irritability of the skin to bacterial irritants in general. No inhibition of the toxic action of tuberculin on the leucocytes of these animals as a result of treatment with oestrogen was demonstrated.

These somewhat conflicting findings serve to illustrate the vastness of the problems involved but suggest that, in the main, variations in sensitivity of the skin are not necessarily accompanied by corresponding changes in the sensitivity of the tissue cells.

#### The present study:

In the chapter on analysis of the data obtained in this investigation an attempt was made to explore the possibility of correlation between the degree of skin sensitivity and the degree of sensitivity of the white cells. As will be remembered, the skin sensitivity of each patient was grouped, for convenience and clarity, according to the size of the threshold reaction. Also since the cytotoxic action of tuberculin was best illustrated numerically in the response of the neutrophil polymorphs, the/



the figures for this group were taken for comparative purposes.

The correlation graphs (Figs. 4 and 5) indicate that in this series no relationship could be demonstrated between the response of the two types of tissue, the skin and the neutrophil polymorphs, to tuberculin.

Nevertheless it is interesting to note that in subject 5 in whom two series of observations were made, first during a stage when what is commonly regarded as a clinical manifestation of a high degree of allergy, phlyctenular conjunctivitis, was present and later, fourteen days before death, when extreme emaciation was present, diminution in the skin response so often demonstrated at this stage in the disease appeared to be mirrored in the response of the leucocytes. It is realised that this is a single observation, and also to be remembered is that the error of interpretation in this series of slides is likely to be greater than in any other owing to the fact that a large number of atypical cells were present, presumably due to a frenzied call on the bone marrow in the presence of an overwhelming and terminal infection.

The action of tuberculin on the lymphocytes is slight, at least under the conditions of the present experiment, and it was not considered, therefore, that any further information would be obtained by plotting graphs with the figures relative to the lymphocytes./



lymphocytes.

Further points of consideration in relation to the skin response:

J. S. Howe (1933) has in an interesting study established a correlation between the skin response to tuberculin and the state of the peripheral blood vessels as measured by the diastolic blood pressure. There was definite evidence also that vasodilation and vasoconstriction reflected in the blood pressure readings were largely influenced by the metereological environment and that, therefore, fluctuations in metereological conditions could cause variations in the skin response.

The liberation of histamine in the skin, which is a factor in the inflammatory reaction of this organ, is another variable factor to be considered when the use of antihistamine drugs is becoming more widespread.

These variable factors may help to explain the lack of correlation between the local and systemic response to tuberculin demonstrated in this study.

It would seem, then, that until further work clarifies the position, the skin, subject as it is to inconstancy of temperature and circulation, does not necessarily under all circumstances mirror accurately the sensitivity of the tissues.

Nevertheless, the accessibility of the skin and the ease with which its response can be measured is a factor which goes far to outweigh any possible inaccuracy/

accuracy the use of it may carry in measuring the sensitivity of the tissues to antigenic substances.

It is, of course, realised that the tuberculin skin test is used principally, not to measure the degree of sensitivity present, but to detect the presence of tuberculin sensitivity with its implication of past tuberculous infection.

VI.

CONCLUSIONS

- (a) The disintegration of the neutrophil polymorph-nuclear cells of the blood of a tuberculous individual is accelerated (when clotting is prevented) in vitro by the addition of tuberculin.
- (b) This acceleration is not related to the degree of skin hypersensitivity to tuberculin demonstrated in the same individual.
- (c) The young polymorph is more susceptible to tuberculin than the more mature cell. This is a tentative conclusion and the subject is worthy of further investigation.
- (d) The lymphocytes of a tuberculous individual (within the conditions of this experiment) are much less susceptible to damage by tuberculin.
- (e) The action of tuberculin on the monocytes of a tuberculous individual cannot be accurately determined because the number of these cells is too small.
- (f) Tuberculin causes swelling and disappearance of the granules, with eventual disintegration of the nucleus of the neutrophil polymorph from the sensitised body.
- (g) Owing to the possibility of unavoidable error, a method involving the differential counting of leucocytes would not appear to be entirely suitable for measuring accurately the cytotoxic effect of tuberculin on the white blood corpuscles.
- (h) A method suitable for measuring the cytotoxic action of tuberculin on blood leucocytes in the laboratory/

laboratory of the average hospital has not been evolved.

(i) Heparin in the dilutions used is suitable for an investigation of this nature involving the incubation of blood and the examination of the leucocytes.

(j) If the action of tuberculin on the neutrophil polymorphs is taken as indicating, at least in some part, the systemic response of the body, then the local and systemic responses to tuberculin do not necessarily correspond.

(k) Measurement of the action of tuberculin on tissue cells would probably be a more reliable index to tissue sensitivity than the response of the skin, as it is independent of factors such as circulation known to alter the response of the skin to excitator substances.

(l) This finding may throw some light on the variable results obtained when an attempt has been made to correlate changes in skin sensitivity with the clinical course of the disease in tuberculous patients. Fluctuations in the skin reaction, which take place in the course of the disease, may not necessarily be accompanied by changes in systemic tissue sensitivity.

(m) The ease of performance, however, of the intracutaneous tuberculin test is of considerable importance in assessing its value as a measure of tissue sensitivity.

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PART II

CLINICAL INVESTIGATION

A CLINICAL EVALUATION OF THE USE OF  
PARA-AMINOSALICYLIC ACID IN THE TREATMENT  
OF PULMONARY TUBERCULOSIS.

Chapter 5PART IIINTRODUCTION

The search for a chemotherapeutic agent effective against the tubercle bacillus with its peculiar physical properties has for long occupied the minds of tuberculosis workers. Following the dramatic discovery of the sulphonamide group of compounds so effective against many previously resistant bacteria hopes were raised that a related substance would be of use in the treatment of tuberculosis. Many drugs belonging to the sulphone group have been tried but have not fulfilled in subsequent trial on human subjects the promise they may have shown in vitro and in animal experiment.

CHEMISTRY AND IN VITRO ACTIVITY OF P.A.S.

In 1940 Bernheim showed that benzoic acid and salicylic acid (2 hydroxy-benzoic acid) increase the oxygen consumption and the carbon-dioxide production of the tubercle bacillus. In the assumption that these acids are oxidised as metabolites and that, therefore, similar compounds might play a part in the metabolism of the bacillus Lehmann (1946) was stimulated to investigate the properties of a large number of derivatives of benzoic acid in the hope of finding a substance, which by virtue of inhibiting the stimulating action on the bacillus, would have bacteriostatic properties for the tubercle bacillus.

Among the first compounds tried, 4 amino-hydroxy-benzoic acid (para-aminosalicylic acid) was found/



found to be the most active.

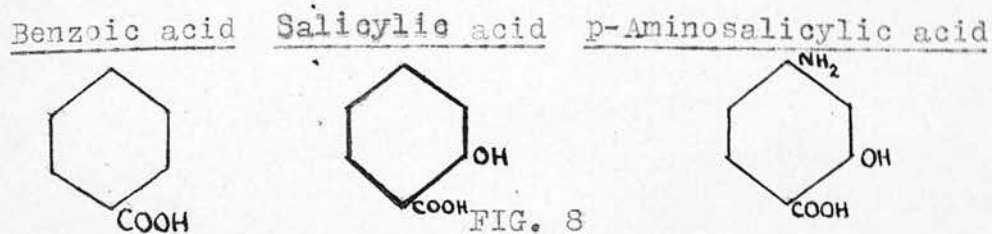


FIG. 8

Chemical formulae of benzoic acid, salicylic and p-aminosalicylic acids illustrating the close chemical relationship.

In his investigations Lehmann found that an in vitro concentration of 0.15 mgm. per 100 ml. of p-aminosalicylic acid produced an inhibition of growth of the tubercle bacillus (B.C.G. strain) of 50-75 per cent. Related compounds were either much less powerful in their action or were toxic for animals.

#### ANIMAL EXPERIMENT

Having found a substance which in vitro was tuberculostatic the next step was to investigate its properties in animal experiment. The compound was administered intravenously, intramuscularly and orally to rabbits and was found to be well tolerated, rapidly excreted in the urine, and, when given by mouth, rapidly absorbed.

Unfortunately in Lehmann's experience the drug was not well tolerated by guinea pigs, the animal most suited to the study of the action of chemotherapeutic agents in progressive tuberculosis. However, even in this worker's experiment where the animals died in five to seven days, apparently from the/

the toxic action of the drug, the beneficial effect on the tuberculous disease in these animals appeared to be demonstrated.

In the experience of Feldman et al (1947), who investigated the properties of this drug in American guinea pigs will tolerate P.A.S. to the extent of 4% in the diet. They have successfully demonstrated the inhibitive properties of the drug in guinea pigs inoculated with a virulent strain of tubercle bacilli six weeks before the start of treatment.

Similarly Youmans et al. (1946), who have conducted an exhaustive series of experiments into the bacteriostatic properties of P.A.S. and related compounds with bacilli of different types and virulence, have demonstrated the bacteriostatic properties of P.A.S. in vitro and also the suppressive effect of the drug on experimental tuberculosis induced in mice by the intravenous injection of 0.1 mgm of the H37Rv strain of tubercle bacilli.

#### CLINICAL TRIALS

Thus the action of this substance on the tubercle bacillus in vitro and in vivo appeared undoubted. There only remained to be answered the all important question. What would be the action of this substance in human tuberculosis?

Gradually knowledge as to its action is being built up as the number of cases treated with P.A.S. grows. The majority of the reported studies have, up to the present been of cases treated in Scandanavian/

Scandinavian and other European countries where the drug has been more freely available for some time. Vallentin (1946) was the first to report the results of treatment in a large number of cases (80) of pulmonary tuberculosis. The drug was given orally in divided doses and a daily total of 14 gm. No toxic reactions were noted and general improvement occurred in most patients during treatment. In some, cessation of treatment was followed by a relapse, but improvement again supervened when a fresh course was instituted.

A limited number of cases (19) were reported by Dempsey and Logg (1947), six of them being the subjects of purely pulmonary tuberculosis, the others having complicating lesions. Again promising results were obtained, the dosage not being stated.

A further interesting series has been recorded by Erdei (1948) although full reports were available for only a limited number. The dosage used initially was 12 gm. per day but later as recorded in a second report the dosage was increased to 20 -25 gm. per day, when the drug became more freely available. Again promising results were obtained and particular emphasis was laid on a striking reduction of toxæmia in the severely ill case. Some patients had been rendered suitable for further surgical measures but with others a state of chronicity had been reached, which in itself was an achievement. This occurrence, however, raised a serious problem in/

in the future management of such cases.

In a more recent series of ten cases reported by Joules and Nassau (1949) improvement was noted in half the number, all of whom were the subjects of progressive bilateral disease in which steady deterioration had been taking place before treatment began. Such a series of advanced cases poses an almost impossible task for any chemotherapeutic agent and the fact that improvement was recorded at all is a point worthy of note. The writers concluded, that despite their relatively disappointing results, further investigation into the therapeutic value of P.A.S. was justified.

#### MODE OF ACTION

It will be remembered that the starting point of Lehmann's work was the discovery by Bernheim that benzoic acid and salicylic acid stimulated the respiration of the tubercle bacillus. His object, was, therefore, to introduce into the benzoic and salicylic acid molecules chemical groupings which would inhibit this metabolic process and thus inhibit, at the same time, the proliferation of the bacillus. Having discovered the substance para-aminosalicylic acid and having proved its undoubted tuberculostatic properties, he set out to demonstrate the inhibition of the stimulating effect of salicylic acid on the respiration of the bacillus which he expected that P.A.S. would have. Contrary to these expectations, however, it was found that/

that P.A.S. had a similar stimulating effect on the bacillus when allowed to act alone. Also P.A.S. will inhibit the growth of the apathogenic B.C.G. strain which has no salicylic acid enzyme system in the same manner as it inhibits the growth of a virulent strain which does contain a salicylic acid enzyme system.

Lehmann therefore concluded that P.A.S. struck more deeply at the metabolism of the bacillus and suggested that the bacteriostatic action of P.A.S. was due to a disturbance of the protein metabolism of the bacillus, perhaps in the nature of an inhibition of the de-amination of amino-acids, which is known to be the effect of benzoic and salicylic acid on cellular metabolism in the animal organism.

Youmans et al (1947) suggest that P.A.S. changes the host's tissue reaction from necrotic exudative to fibrotic proliferative and at the same time exerts a depressive action on the tubercle bacilli. Erdei (1948) believes the action of P.A.S. in the tuberculosis patient to be complex; the response to the drug illustrating both the direct bacteriostatic effect of the drug on the bacillus and the direct pharmacological action of the drug on the host, perhaps as a result of its close chemical relationship to the salicylate group.

Thus we have no certain evidence, substantiated by experimental proof, of the special attribute of P.A.S. which is responsible for its action on the tubercle/



tubercle bacillus both in vitro and in vivo. We are then unable to say why exactly, P.A.S. should have a beneficial action in tuberculosis and until further research clarifies the matter, this fact may well hinder the search for a related compound with a still more powerful action in the treatment of tuberculosis.

#### PHARMACOLOGY

Para-Aminosalicylic acid is administered orally in the form of the sodium salt. The drug is now universally available in the form of sodium para-aminosalicylate dihydrate which is a slightly crystalline substance readily soluble in water. In solution it is a light brown liquid, darkening slowly on standing, and with a bitter taste.

The drug is unstable in solution and solutions should be freshly prepared every two or three days.

When administered orally rapid absorption and excretion take place, excretion being by the kidneys. In an investigation carried out by Erdei (1948) blood levels of around 8 mgm. per cent were reached 15 minutes after ingestion of 2.0 gm. P.A.S. and up to 11 mgm. per cent with 3.0 gm; however, in 3 hours, the blood concentration fell to 3 mgm. per cent and below. 24 hours after the withdrawal of the drug the urinary concentration fell almost to nil. Similar results are described by Way et al (1943). If the drug is given orally 50 per cent to 80 per cent recovered from the urine is in the acetylated/

acetylated form. No storage of P.A.S. has been demonstrated anywhere in the body but high concentrations are temporarily attained in the kidney, the lung and the liver and appreciable amounts are bound by the plasma proteins, (Way et al, 1948).

The rapid excretion of the drug, necessitates frequent doses and work still continues to determine the optimum scheme of dosage for all patients.

## Chapter 6

### PRESENT STUDY

The following is a presentation of the results obtained in twenty cases of pulmonary tuberculosis treated with para-aminosalicylic acid. It includes an extended study of ten cases, the preliminary findings from whom were presented to the Tuberculosis Society of Scotland as a contribution to a Symposium on the treatment of tuberculosis with para-aminosalicylic acid on January 28th 1949.

### PLAN OF TREATMENT

#### Type of case

All the cases chosen for treatment with P.A.S. were the subjects of bilateral pulmonary tuberculosis of varying extent. Fourteen of the cases had advanced disease; in four of these the lesions were predominantly unilateral with a recent spread to the contralateral lung. In ten, cavity formation was established, and in some multiple, while in the remainder, signs of early softening were suggested, both radiologically and clinically.

A further four cases, the disease being less extensive, were grouped in the intermediate category, and in two of these cavity formation was present at the commencement of treatment. In the remaining two cases the infiltrations were small, although present bilaterally. They have been classed as early.

In none of the patients was the disease of the chronic/

chronic fibrotic type and, although the duration of the disease could not in each case be accurately determined, it appeared radiologically to be of fairly recent origin. Although in all the cases exudative disease which, it was thought, would benefit from chemotherapy was present, the ultimate decision to treat each individual case with P.A.S. was made for a variety of reasons. In some it was with the hope that chemotherapy would bring them later within the bounds of further surgical measures. In others it was used with the object of controlling contralateral disease to allow of surgical collapse of the principally involved lung, and, yet again, in others where simple measures had failed to produce quiescence of the lesions.

There were also considerable variations in the length of sanatorium treatment before the start of chemotherapy. With seven patients, in whom strong indications for the use of the drug were present, the drug was commenced soon after admission, immediately investigations were complete. But with the remainder the period varied from over two years to one month.

Full case summaries detailing and elaborating this information for each case will be presented later.

#### The problem of controls

In a disease such as tuberculosis where there are so many factors varying between individuals, the controlled/

controlled study of the therapeutic efficacy of any one form of treatment is, outside the experimental laboratory, an ideal extremely difficult to achieve. With only a relatively small patient population to draw from it is an impossibility, and no attempt has therefore been made in this study to control the results obtained in the recognised scientific manner. But in assessing the results an attempt has been made to estimate the actual progress achieved gauged against the expected progress of the disease with regard to the trend observed before treatment started. Thus a form of control, albeit an arbitrary one, is available.

#### Dosage

The administration of a drug such as para-amino-salicylic acid absorbed, as it is, quickly, and excreted rapidly from the body, presents certain problems.

When this inquiry was begun experience and available evidence was limited. In reported studies dosage had varied between 10 gm. and 15 gm., in divided doses, daily, but experience seemed to indicate that a higher dosage was desirable.

Consequently it was decided to follow initially the scheme of dosage as recommended by Herts. Pharmaceuticals in their early literature. This consisted in 3 gm. doses given at  $2\frac{1}{2}$  hourly intervals during the day with a 5 gm. initial morning dose. On the first two nights of a six day schedule/



schedule 5 gm. doses were given twice in the night. A complete rest from the drug was given one day in every seven.

After only a short trial of this scheme many patients exhibited signs of intolerance and volunteered the information that it was the night doses which they found most disagreeable. A change was therefore made, retaining the  $2\frac{1}{2}$  hourly interval but eliminating the night doses. Seven doses were thus given daily and by increasing the first and the last dose each day to 5 gm. the daily intake was raised to 25 gm. The principle of one day's rest in seven was also retained, mainly because of the patient's reaction to the weekly prospect of a day's rest, which, it was thought, would promote their continued cooperation in a prolonged and, perhaps slightly unpleasant, mode of treatment. Thus the weekly intake on this scheme of treatment was 150 gm.

Tolerance to the drug varied from patient to patient and a few found the amended dosage troublesome. In four, therefore, the dose was reduced to 15 gm. per day (5 doses of 3 gm. at  $2\frac{1}{2}$  hourly intervals). This dosage was tolerated well. In others elimination of the late evening dose was sufficient to eradicate the toxic effects, and in those the dosage was maintained at almost the original level by distributing the drug evenly throughout the day, giving 4 gm. six times at  $2\frac{1}{2}$  hourly intervals.

Preparation/

### Preparation of drug

Shortly after this enquiry began it was learnt that the majority of our colleagues who were utilising the drug in Scotland were employing the Herts. Pharmaceuticals preparation. At this stage, therefore, it was decided to change over to the Ward, Blenkinsop preparation of the drug in order to determine whether or not there was any difference in the therapeutic efficacy of the two substances. Later a change was made back to the Herts. Pharmaceuticals preparation so that in this series 15 patients were treated with the Ward, Blenkinsop & Co., preparation and five with the Herts. Pharmaceuticals preparation.

### Dispensing

From both firms the drug was supplied in the form of the crystalline sodium dihydrate salt. This was dissolved in water and for the convenience of the nursing staff a concentration of 1 gramme to 1 fluid drachm was used.

A variety of flavouring agents were tried but the patients preferred to take the drug in the simple solution, followed immediately by a longer drink either of glucosade, or water, plain or flavoured with fruit juices.

### Toxic Effects

The toxic effects of the drug exhibited in this series of patients were limited to nausea, vomiting and diarrhoea. If these were not controllable by simple/

simple remedies the dose was reduced and this was usually sufficient to produce the elimination of these symptoms. In only one case was apparent complete intolerance shown. This was manifest immediately on starting the drug and consisted in severe, uncontrollable vomiting and diarrhoea which necessitated the abandonment of the course. The case was one of advanced pulmonary tuberculosis with marked toxæmia. Steady and rapid deterioration followed the cessation of the drug but it was not felt that sufficient had been given (39 gm. were given in all) for the deterioration to be attributable to the drug, if indeed the violent disturbance which followed the onset of treatment was truly a sign of intolerance. This case has not been included in the analysis of results.

In an attempt to avoid toxic effects some patients, at the commencement of their course, were given the drug in gradually increasing doses in the first few days but this resulted in no appreciable difference. It was found that if the patient was tolerant to the drug full doses could be tolerated immediately, and, in those in whom toxic effects were exhibited, an accumulative action appeared to take place. As a result any vomiting or diarrhoea was more often manifest towards the end of the six day course and the day's rest at the end of this time would commonly be sufficient to arrest it.

The drugs used in the control of the toxic effects/

effects were a simple chalk and opium mixture, a kaolin preparation and a bismuth mixture. Vit. B. was also tried for the control of the intestinal upset but without apparent benefit.

No patient showed at any time any evidence of oedema, urinary disturbance or cardiac irregularity which have been described as toxic manifestations of the drug (Personal communication; Lehmann 1946).

There was no noticeable difference in either the incidence or severity of the toxic effects between the preparations obtained from the two different firms, Herts. Pharmaceuticals and Ward, Blenkinsop & Co.

#### Length of course

Again when this enquiry began, insufficient was known regarding the clinical use of the drug for a complete schedule of treatment to be definitely laid down. It was decided to give the patients originally chosen a minimum of three months treatment and at the end of that time review each case with a view to any benefit achieved. Following this three months trial it appeared that in the majority of cases a longer period was necessary and in those patients who had shown a degree of improvement, with the possibility of further benefit, a second course was given. This principle of reviewing each case at the end of three months treatment was followed with all cases and in all the degree of benefit, although in some perhaps slight/

slight, was sufficiently promising for the treatment to be continued.

To summarise the position for the group of patients under study:

Of 15 patients whose course is completed:

4 patients have had 12 weeks treatment

2    "            "            "    20    "            "

5    "            "            "    22    "            "

4    "            "            "    24    "            "

Of 5 patients whose treatment is continuing at the time of writing:

2 patients have had 18 weeks treatment

1 patient has had 17    "            "

1    "            "            "    15    "            "

1    "            "            "    12    "            "

All patients were kept at rest in bed during the course of treatment, although those whose condition permitted were allowed up for toilet purposes.

In two cases, detailed later, a course of streptomycin was given together with the P.A.S. for a period during the P.A.S. course.

#### Estimation of para-Aminosalicylic acid in blood

Throughout the course estimations of the P.A.S. blood level attained was carried out in each patient, weekly in the first instance, and later at fortnightly intervals. Several recommended methods were tried in order to determine which would prove the most reliable and also most suitable for use with the comparatively limited laboratory facilities available/



available in the average hospital laboratory.

Method I - as recommended by Herts.

Pharmaceuticals.

Reagents:

1. 10% trichloroacetic acid

2.  $N/4$  sodium hydroxide

3. Ehrlich's reagent:- prepared as follows -  
3 gm. paradimethylaminobenzaldehyde is dissolved in  
3 ml. concentrated sulphuric acid, and added to  
100 ml. distilled water.

(Note - As Ehrlich's reagent required to be freshly prepared for each batch of estimations it was found in practice that a more satisfactory solution was obtained if a mixture of 1 ml. concentrated sulphuric acid in 33 ml. distilled water was added to 1 gm. paradimethylaminobenzaldehyde and the dissolving hastened by gentle heating, as, for instance, by immersing the beaker in warm water.)

#### Procedure

2 ml. blood obtained by venepuncture and oxalated (potassium oxalate) to prevent clotting is added to 3 ml. of 10% trichloroacetic acid, thoroughly mixed and filtered, a clear solution being obtained.

To 4 ml. of the filtrate 3 ml. of  $N/4$  sodium hydroxide is added. The addition of 1 ml. of Ehrlich's reagent is then made and the reading on a photo-electric colorimeter taken immediately; comparison being made with a blank containing distilled/

tilled water in place of the blood. In taking the colorimeter reading a blue light filter is used.

A calibration curve is compiled from the colorimeter readings obtained from solutions containing concentrations of P.A.S. of 20, 10, 5 and 2.5 mgm. per 100 ml., these solutions having been treated as for a similar quantity of blood as above. On this curve the colorimeter reading obtained after treatment of the blood specimen is plotted, and an estimation can thus be made of the concentration of P.A.S. in the specimen.

The disadvantages of this method in practice are as follows:

1. In the compiling of the standard graph it was found that, particularly with the higher concentrations of P.A.S., an orange precipitate formed very quickly in the final solutions. This obviated any reading obtained and it was necessary therefore to take the reading immediately on the addition of the last reagent.

2. The range of colours obtained (yellows) was very narrow particularly in the range of concentration found in the blood samples. A completely satisfactory curve was not therefore procured and increased the possibility of inaccuracy in the estimation of the level obtained.

3. Different batches of paradimethylaminobenzaldehyde obtained from the same manufacturer appeared to vary in the intensity of the colour obtained when/

when in solution and to a slight degree in solubility. This rendered inaccurate the estimation of blood levels when using the original standard graph and necessitated the compiling of a graph from a fresh set of standards for each batch of this substance. The advantage of this method lies in its relative ease of performance.

### Method II

This method as described by Klyne and Newhouse (1948) also employs Ehrlich's paradimethylaminobenzaldehyde reagent but with considerable modification of method which gives it some advantages over the previous one.

### Reagents:

1. p-Toluenesulphonic acid, 20 gm. in 100 ml.  
0.2N Hydrochloric acid
2. Disodium hydrogen citrate solution, 0.75M  
(39.4 gm. A.R. citric acid, dissolved in 188 ml. 2N sodium hydroxide and diluted to 250 ml.)
3. Paradimethylaminobenzaldehyde, 2 gm. A.R. in  
95% ethanol (100 ml.)
4. Sodium para-aminosalicylate standards

### Procedure

0.5 ml. oxalated whole blood is added to 6.5 ml. distilled water, shaken and stood for five minutes to lake. The proteins are then precipitated with 3 ml. p-toluenesulphonic acid added slowly. After being allowed to stand for five minutes the mixture is filtered (Whatman No. 40 or 42 paper)/

paper).

To 5 ml. of the filtrate (which must be clear) are added 1 ml. of the citrate buffer solution and 2 ml. of the 2% paradimethylaminobenzaldehyde reagent.

As before the colour which develops is measured on a photo-electric colorimeter, using a blue light filter, against a blank containing distilled water. The blood P.A.S. level is then estimated by means of a previously compiled standard curve.

This method eliminates a fault of the previous one, namely precipitation, and thus the colour obtained is stable for a number of hours. Again however a narrow range of colours results, giving an unsatisfactory calibration curve.

It also cannot be used when other primary aromatic amino-compounds (e.g. sulphanilamide derivatives) are likely to be present.

### Method III

This method was suggested by Ward, Blenkinsop and Co. Ltd. and employs the coupling of a diazotised solution of sulphanilic acid with an alkaline solution of P.A.S.

The method was originally supplied in outline and was elaborated with the assistance of Professor Kermack, Royal College of Physicians Laboratory, Edinburgh. The procedure was employed in the greater part of this study and is as follows:

### Reagents:

1./

1. 20% Trichloroacetic acid.
2. Sodium hydroxide 100° TW. (53 gm./litre W/V.)
3. Diazotised sulphanilic acid (To 50 mgm. sulphanilic acid, dissolved in 5 ml. water with hydrochloric acid to assist solution, is added sufficient  $\frac{1}{2}\%$  sodium nitrite solution to obtain a positive test with starch iodide paper. This solution is freshly prepared and kept cool in iced water for each estimation of P.A.S. concentration)

#### Procedure

10 ml. oxalated venous blood is centrifuged at 2,500 revs. per minute for 15-20 minutes. The clear serum is pipetted off and  $\frac{1}{5}$  of its volume 20% trichloroacetic acid added. The resultant precipitated solution is again centrifuged for 15-20 minutes and 2 ml. of the clear deproteinated fluid taken. To this is added 0.5 ml. sodium hydroxide and finally 0.5 ml. diazotised sulphanilic acid. A reddish-brown colouration develops and darkens slowly on standing. This necessitates that the colorimeter readings be taken at a stated interval following the addition of the last reagent, and as before a reagent blank containing distilled water is used. A green light filter is recommended for use in this estimation.

In order to obviate the inaccuracy resultant upon the instability of the colours obtained, calibration curves were drawn up for standard solutions/



solutions of P.A.S. at intervals of 5, 10, 20 and 30 mins. following the addition of the last reagent. Any of these were then available for the interpretation of readings should an interruption render the figures at a stated interval invalid. Care had also to be taken that the strength and measurement of the sodium hydroxide solution was kept constant as the intensity of the colour obtained is dependent on the pH of the final solution.

Dickensen and Kelly (1949) have recently described their elaboration of this method which they claim to have given satisfactory results. It is as follows:

#### Reagents:

1. 10% trichloroacetic acid
2. 30% sodium hydroxide
3. Diazotised sulphanilic acid. Sulphanilic acid solution (1% sulphanilic acid in 10% hydrochloric acid) is cooled in ice and 10% silver nitrate solution added until the reaction is just positive to starch-iodide paper; then a little sulphanilic acid solution is added until the starch-iodide test is negative. The solution is kept cold (0-5°C) and made up fresh for each batch of tests.

#### Procedure

2 ml. of blood serum is obtained from oxalated venous blood by centrifuging. This is diluted with 2 ml. water and a further 2 ml. 10% trichloroacetic/

cetic acid added to precipitate the proteins. This mixture is filtered (Whatman No. 42 paper) and a clear solution obtained. 2 ml. of the filtrate is made strongly alkaline by the addition of 0.25 ml. of 30% sodium hydroxide after which 0.25 ml. of the diazotised sulphanilic acid is added. A red-brown colour is obtained and the intensity is estimated as before in a photo-electric colorimeter.

A trial of this modification revealed no striking advantage over that elaborated in this laboratory.

Both methods, however, are a distinct improvement over the first two described in that the colour range is wide and in consequence a good calibration curve can be drawn.

#### Method IV

As a result of a personal communication with Dr. W. Klyne, London Postgraduate School of Medicine, the description of a further method of estimation was obtained. This employs the diazotisation of the primary aromatic amine group followed by coupling with naphthylethylenediamine.

#### Reagents:

1. Trichloroacetic acid 25% (W/V)
2. Sodium nitrite 1% (W/V)
3. Ammonium sulphamate 2 gm./100 ml. 50% glacial acetic acid (Analar)
4. N - 1 - naphthylethylenediamine hydrochloride 0.2%
5. Concentrated hydrochloric acid.

Procedure/

## Procedure

0.2 cc. oxalated whole blood is measured into a test-tube containing 6.7 ml. distilled water, shaken and stood for 3 mins. to lake. 0.6 ml. 25% trichloroacetic acid is then added and after shaking the mixture is stood for 20-30 mins. for the protein precipitate to settle. It is then filtered through a No. 42 Whatman filter paper in a 1 inch diameter glass funnel.

To 5 ml. of the filtrate (which must be clear) are added 1.5 ml. conc. HCl and 0.2 ml. of the sodium nitrite solution. The tube is shaken for 30-40 sec. and 1.0 ml. of the ammonium sulphamate reagent is added immediately. The solution is shaken for 10 sec. to free it from nitrogen bubbles, then 1.0 ml. of the naphthylethylenediamine solution is added. (The timing and shaking at these stages must be uniform).

In the presence of P.A.S. a purple colour develops which is stable and the concentration of P.A.S. can be estimated as before by comparing the colorimeter reading obtained (green filter) against a reagent blank with a standard curve previously prepared.

This method has two important advantages, viz. the colour developed is stable and is directly proportional to the P.A.S. concentration from 0 - 10 mgm. per 100 ml. of blood. The colour range is satisfactory although not so wide as that obtained in/

in Method III.

That so many methods have been described for the estimation of P.A.S. in body fluids is an indication that no completely satisfactory method has yet been elaborated.

With only the resources of a small hospital laboratory at our disposal we have come to the conclusion that, of the four methods tried, the most wholly satisfactory is Method IV. It is simply executed and it would appear to offer results with a fair degree of accuracy.

#### Blood levels obtained

Owing to the rapid excretion of the drug as previously mentioned there are two variable factors which will alter appreciably the figure obtained for the level of the drug in the blood at any one time. These are the size of the dose, both the total and individual, and the time in relation to the previous dose that the specimen of blood is taken. Variations in the figures obtained in this investigation were easily traceable to these facts, as, it will be remembered, certain patients were given doses of 4 gm. 6 times daily, other 3 gm. 5 times with or without two doses of 5 gm. And also it was impossible to keep constant the time of removal of the blood.

The figures obtained varied between 2.5 and 10 mgn. per cent.

The average level obtained for each patient throughout/

throughout their course of treatment varied from 3 mgm. per cent to 7 mgm. per cent. The average figure for 16 patients receiving 24 or 25 gm. daily was 5.7 mgm. per cent; the figure for those receiving 15 gm. daily was 3.9 mgm. per cent.



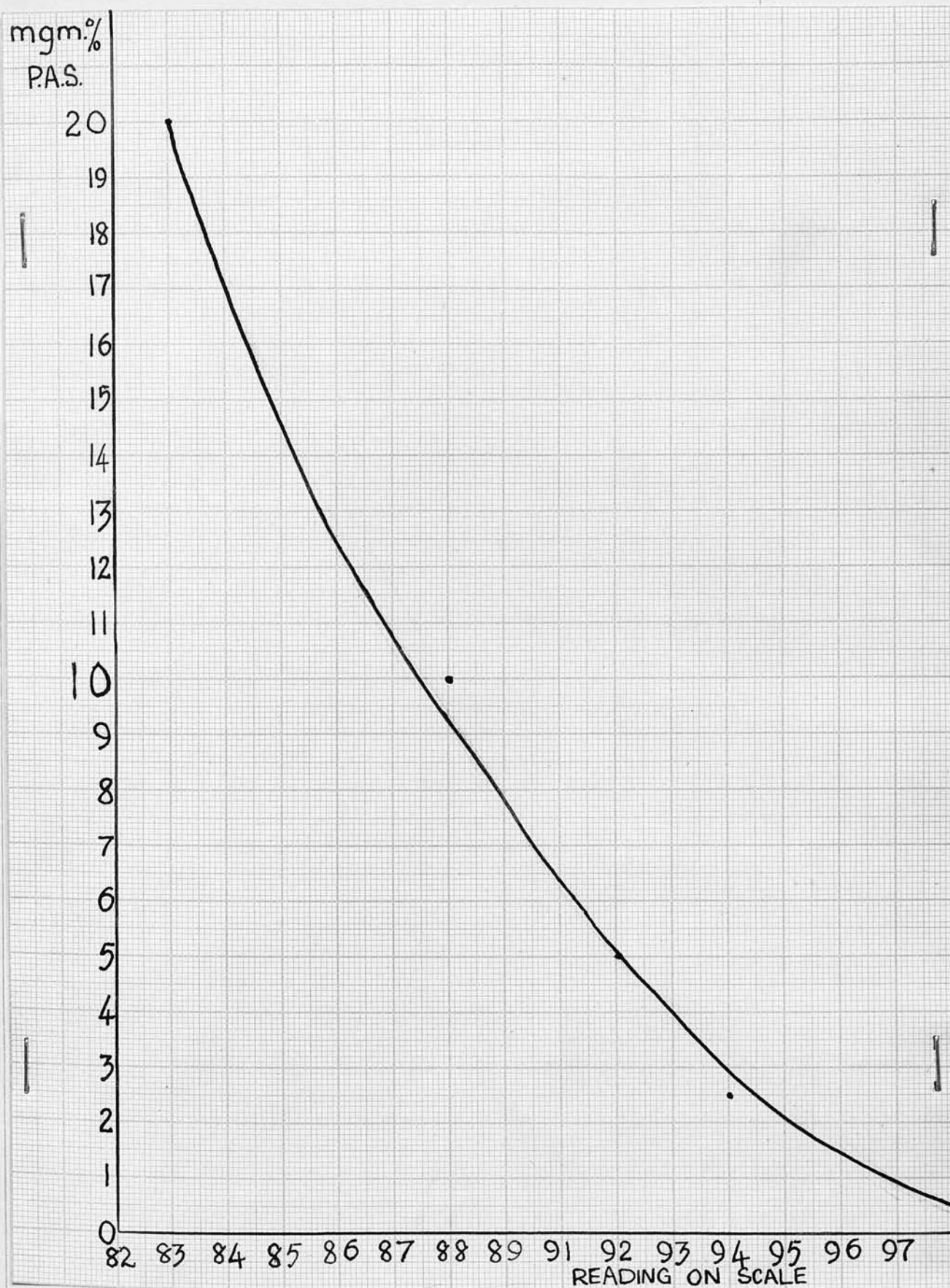


FIG. 9.

Calibration curve for standard solutions  
of P.A.S. Method 1.

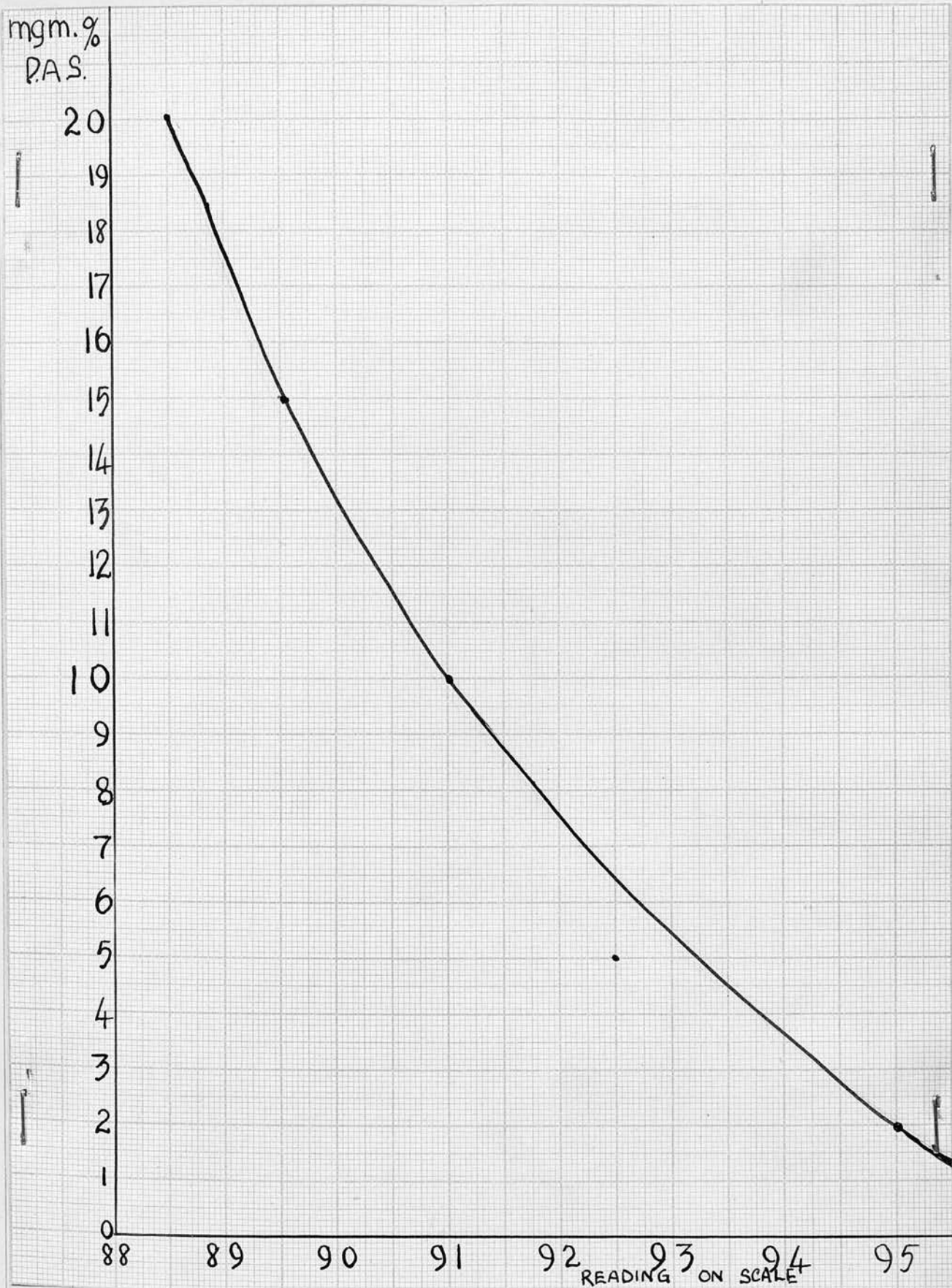


FIG. 10.

Calibration curve for standard solutions  
of P.A.S. Method 2.

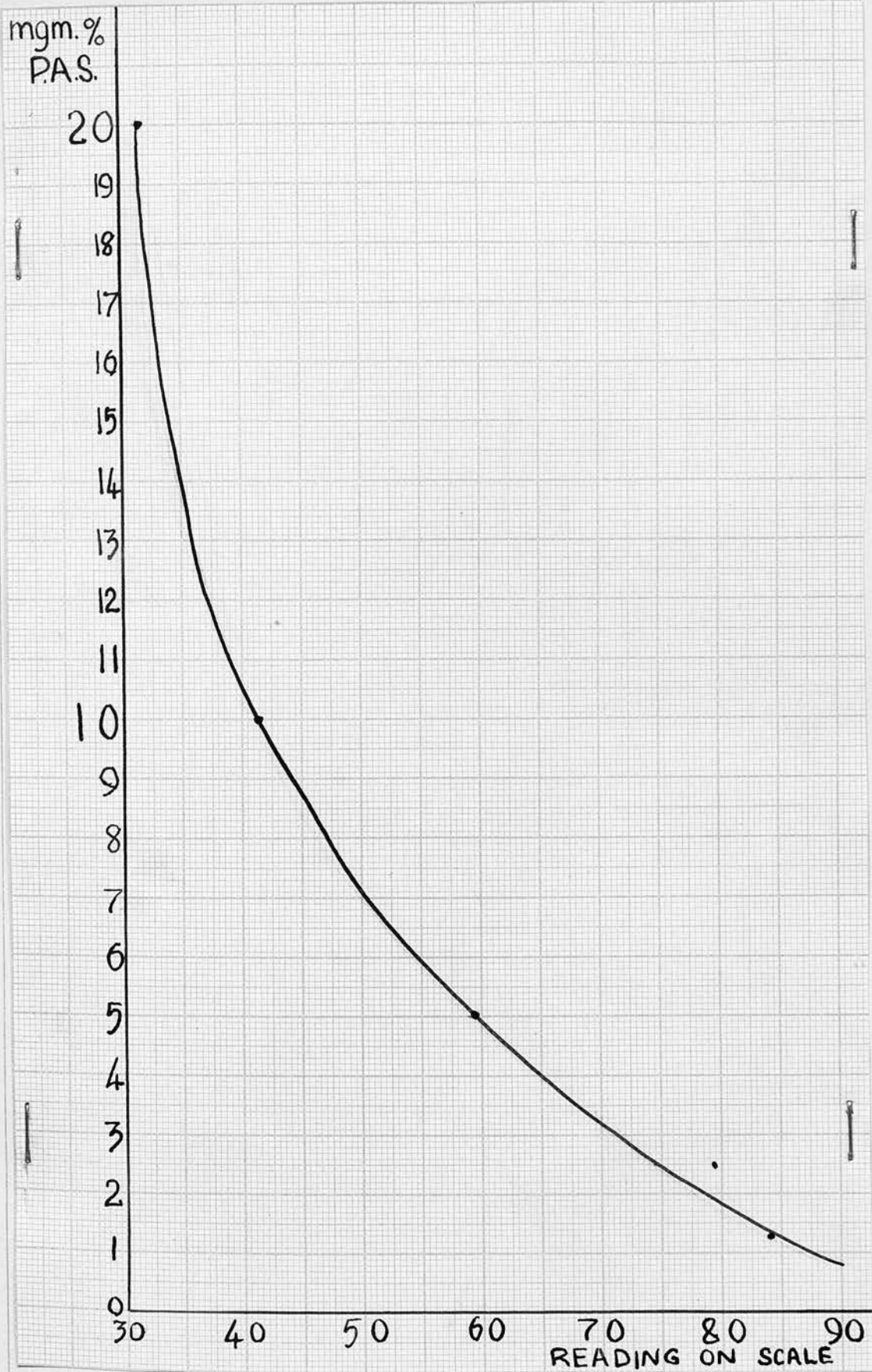


FIG. 11.

Calibration curve for standard solutions  
of P.A.S. Method 3. ( Readings taken 10  
minutes following the addition of the last reagent.)



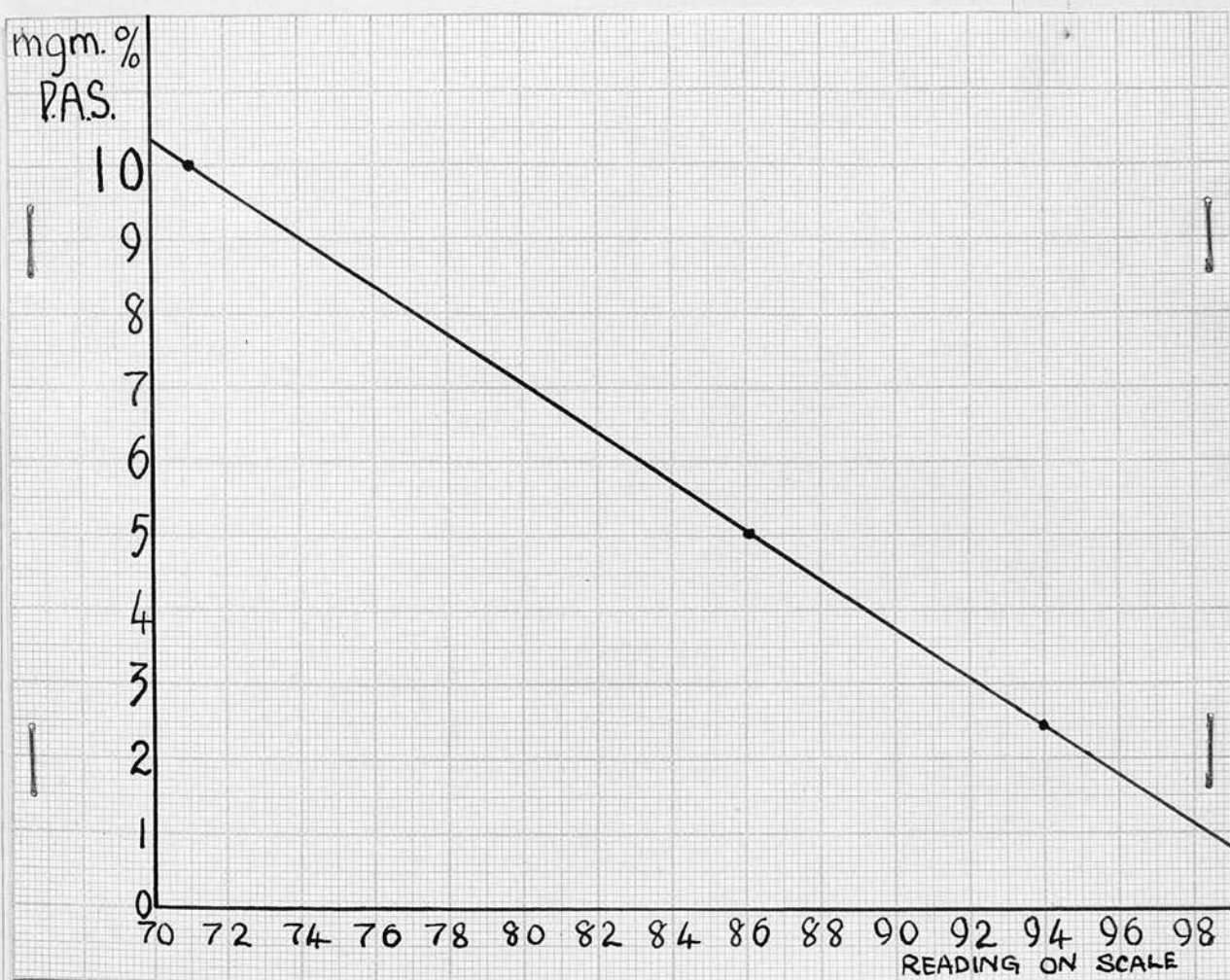


FIG. 12.

Calibration curve for standard solutions  
of P.A.S. Method 4.

Estimation of urine levels

As P.A.S. is excreted wholly and rapidly by the kidney, either unchanged or in the acetylated form, very high concentrations are reached in the urine.

After a series of preliminary estimations of the urine level, during which concentrations varying from 300 mgm. per cent to over 1000 mgm. per cent were recorded, further estimations were omitted. The concentration must of necessity vary with the amount of fluid drunk, the degree of kidney efficiency, and the interval following the dose that the urine is voided. Even if the concentration were estimated on a twenty-four hour specimen great variations are recorded. (Twenty-four hour specimens were used for the above noted estimations).

For these reasons, therefore, it would seem that estimations of the urinary level of P.A.S. have little clinical value.



Chapter 7RESULTS OF TREATMENT

In analysing the results obtained from any form of treatment in pulmonary tuberculosis individual factors vary so greatly that a general summary of the series can only indicate a trend. It is however useful to present a composite impression in order that the whole picture may more easily be assimilated. Detailed consideration of each individual case will be presented later.

Changes can conveniently be divided into local and, general or systemic. The latter will be considered first.

SYSTEMIC CHANGESWeight

One of the more reliable signs, in the absence of anasarca, of the progress of the disease in tuberculosis is alteration in the weight of the patient. Many patients, even with advanced disease, do however tend to gain weight during the first few weeks of sanatorium regime. The fact that some of the patients were placed under treatment with P.A.S. shortly after admission and, therefore, that gain in weight in these cases may partly be due to this, is balanced by the fact that a larger number had been under treatment for some considerable time before commencement of P.A.S. therapy.

This initial period was therefore passed and had already resulted in considerable increase in weight/

weight.

In the period under consideration, i.e. during the period of P.A.S. therapy, thirteen patients gained weight, six of these amounts of 2 stone and over. Six patients lost weight, two of these losing 3 lbs, the rest smaller amounts, and, in several, an initial gain was followed by a subsequent gradual loss. In the remaining patient an initial gain was again recorded but subsequent gradual loss in weight resulted in her original weight being returned to, although there was then no further loss.

Minor fluctuations occurred from week to week as is commonly the case and this was most marked in those where the change was not a striking feature.

TABLE 7

Weight changes of 20 patients during treatment with P.A.S.

TYPE OF CASE	GAIN		LOSS		STATIONARY
	< $\frac{1}{2}$ st.	> $\frac{1}{2}$ st.	> $\frac{1}{2}$ st.	< $\frac{1}{2}$ st.	
Early	0	1	1	0	0
Intermediate	1	1	2	0	0
Advanced	5	5	1	2	1
Total	6	7	4	2	1

Blood/

### Blood Sedimentation Rate

The estimation of the blood sedimentation rate has become a routine procedure in the assessment of tuberculosis, although the exact significance of changes and variations from the normal is still undetermined. At the commencement of treatment, the rate of sedimentation was accelerated in 18 of the patients. In all a marked initial fall in the rate recorded was an interesting feature of the study. This finding has been reported elsewhere (Erdei and Snell 1948) but an adequate explanation of the phenomenon cannot be put forward.

Although the B.S.R. figure in many of these cases continued to fall or to fluctuate around the lower level, in some a gradual rise occurred in subsequent weeks. As is to be expected a persistently low figure was a more favourable feature than a secondary rise.

TABLE 8.

Analysis of B.S.R. figures in 20 patients under treatment with P.A.S.

B.S.R.	Before treatment				After treatment			
	Early	Int.	Adv.	Total	Early	Int.	Adv.	Total
Below 10	1	1	0	2	2	4	5	11
10 - 30	1	2	1	4	0	0	3	3
31 - 50	0	1	2	3	0	0	3	3
Over 50	0	0	11	11	0	0	4	4

### Temperature and Pulse Disturbance

In five cases an appreciable disturbance of temperature was recorded before the start of treatment and in all the effect of the administration of P.A.S. was early manifest. Reduction to within normal limits occurred within a period of two weeks in all but one case. An occasional evening rise in rectal temperature was recorded in a further two patients but this was unaffected by treatment.

The general absence of this evidence of toxæmia in this series of patients is again due to the fact that the majority had already undergone a spell of sanatorium treatment before the commencement of P.A.S. therapy. This is also true of night sweats which were not a feature manifest to any degree.

### Appetite

It has been noted above that the major toxic effect of the drug consisted in intestinal upset. It is understandable therefore that a number of patients should complain of some loss of appetite. It was noticeable that many ate better at the beginning of the week's treatment following the day of rest from the drug, although not necessarily on that day itself. Several also experienced a welcome and noticeable return of appetite when the course of treatment was finished. As is understandable those patients who experienced least in the way of intestinal upset gained weight most satisfactorily during/

during treatment.

### General Condition

During the course of P.A.S. therapy the general condition of 14 patients improved appreciably, the improvement in some being considerable. This conclusion is not merely the result of an analysis of the foregoing features for each individual case, but is also a measure of the general impression gained of the change in the patients' mental and physical health over the period of treatment.

### Tuberculin Skin Testing

Serial Mantoux tests with dilutions of tuberculin from 1:1,000,000 to 1:1,000 were carried out on 17 patients before the start of treatment and again towards the end or on cessation of treatment. In the repeat series of tests increase in the intensity of the reaction to the extent of sensitivity to a higher dilution being obtained, occurred in 6 cases; increase in the intensity of the reaction to the extent of an appreciable increase in the amount of the erythematous reaction and induration to the threshold dilution, occurred in a further 6 cases; no difference in the two responses occurred in 3 cases, and diminution in the amount of the response in 2.

No relationship could be detected between the changes noted here and the response to treatment.

In view of the fluctuation in sensitivity which is known to take place during the course of the/



the disease in tuberculosis the significance of the finding is uncertain but it is interesting that this high proportion of patients should demonstrate an increase in sensitivity.

### LOCAL CHANGES

#### Cough and Sputum

In over half the number of patients in this study cough was a minor feature of their illness, and only very little or no sputum was expectorated in the day. Of the remainder, <sup>in</sup> four patients reduction in the amount of sputum occurred during P.A.S. therapy, while with the others no appreciable change occurred.

#### Bacteriology

In all but two cases tubercle bacilli were demonstrated in the sputum or in the gastric washings at the commencement of treatment. In nine cases culture either of the sputum or gastric washings have since proved negative for tubercle bacilli. This figure includes the two patients in whom tubercle bacilli were not demonstrated at the commencement of treatment.

No special feature was noted in the morphology of the bacilli during treatment.

#### Physical Signs

The physical sign of the greatest significance in this study and present in all the advanced cases was moist accompaniments to the breath sounds. A diminution in the amount of moisture heard took place/

place initially in all cases. Complete disappearance of moisture occurred in only three and in some of the others a return to the original findings occurred after the initial improvement.

#### Changes in Radiological Picture

As a result of the wide variety of clinical problems which make up this series of cases it can be understood that considerable variation also occurred in the radiological changes which took place. To present a true picture in summarised form is impossible and only a careful study of each individual case will yield accurate information.

It is proposed, however, to divide for convenience radiological changes into resolution, fibrosis and cicatrisation, cavity closure, and deterioration.

Each patient was X-rayed at monthly intervals throughout treatment. Careful consideration of these pictures and comparison with those taken before treatment started have led to the conclusions detailed below.

#### Resolution

Clearing of infiltration occurred to varying degrees in a number of patients. This feature is represented in tabular form in Table. 9.

TABLE/

TABLE 9.

Degree of resolution in 20 patients  
treated with P.A.S.

Type of case	Resolution			
	Marked	Moderate	Slight	Nil
Early	0	0	1	1
Intermediate	1	0	3	0
Advanced	4	4	5	1
Total	5	4	9	2

#### Fibrosis and cicatrisation

Fibrosis is a pathological change which is not likely to be manifest to any striking degree in the short period of time occupied by this study. The findings in this group are confined mainly to the recording of condensing and hardening of the individual foci as evidenced radiologically. In two cases the fibrosis and cicatrisation was regarded as of a moderate degree while in nine others a slight degree was recorded.

#### Cavity closure

In three cases cavity closure was proved by tomography to have occurred during treatment. Reduction in the size of cavities was, however, a feature encountered frequently and in some patients was quite a dramatic change. A further point of interest is that, in the cases in which this improvement was shown, the change occurred in the first or second month of treatment. In six cases the/

the improvement persisted to the end of the course but in the other two the reduction was only temporary.

### CASE NOTES

It is now proposed to examine each case individually in order that a more accurate picture may be obtained of the differing problems involved. The cases are presented in chronological order as they came under consideration for P.A.S. therapy. The salient features for each patient will be presented here but further details are available for reference in the appendix to this work.

Case 1. N.M. A young girl aged 15 in whom the presenting symptom was a cough of short duration, approximately one month. The history contained little else of interest apart from a mild attack of pleurisy which had taken place a little over a year previously.

When first seen she was pale and thin, weight 6st. 10 lbs., but other signs of toxæmia were not a feature. Temperature and pulse were within normal limits.

On physical examination of the chest bronchovesicular breathing with moist accompaniments was noted in both interscapular regions and below the left clavicle.

X-ray examination (Fig.13) revealed widespread tuberculous disease in both lungs; scattered mottling being present in all zones of the right lung/

lung densest immediately below the clavicle and in the lower zone. In the left lung the apex, upper and mid-zones were involved and although no clear cut excavation was present in either lung a suggestion of softening was present immediately below the anterior end of the second right rib.

The lesions were considered recent and active. Tubercle bacilli were isolated on gastric lavage, a satisfactory specimen of sputum not being obtainable.

#### Treatment

In the first instance this was confined to bed rest alone and during this period considerable improvement occurred particularly in the general condition. A gain in weight of one stone was recorded. Little change was noted in the radiological appearances however and on 11-10-48, three months after admission, a course of P.A.S. was started.

Length of course: 24 weeks (2 courses of 12 weeks with a month's interval between)

Dosage: 25 gm. per day.

Average blood level obtained: 6.1 mgm. per cent.

Signs of intolerance: nil.

#### Progress

Weight: Gain of 9lbs. during P.A.S. therapy.

B.S.R.: Fall from 47 m.m. in 1st hour at beginning of course to 8 m.m.

Bacteriology: Although tubercle bacilli were no longer found on direct smear of the gastric washings, following treatment the culture was still positive/



positive.

Radiological changes: A steady improvement took place during treatment, gradual resolution of the lesions occurring. The changes took place most rapidly during the first few months of treatment, the tempo gradually slowing down as time passed. A slight degree of fibrosis and cicatrisation was also noted in later months. Figs. 14 and 15 show the appearances at the end of the first course, and at the finish of treatment respectively.

#### Summary

Improvement in general condition

Improvement in X-ray findings.

#### Conclusions

That this patient has a high natural resistance to the tubercle bacillus there can be little doubt but the degree of improvement and the rapidity with which it took place is considered to be greater than that which could be expected to occur with rest alone.

Prognosis: Improved by treatment.

Addendum: 3 months following the cessation of treatment a small cavity was demonstrated by tomography behind the upper pole of the right root shadow. Further treatment will therefore be necessary.

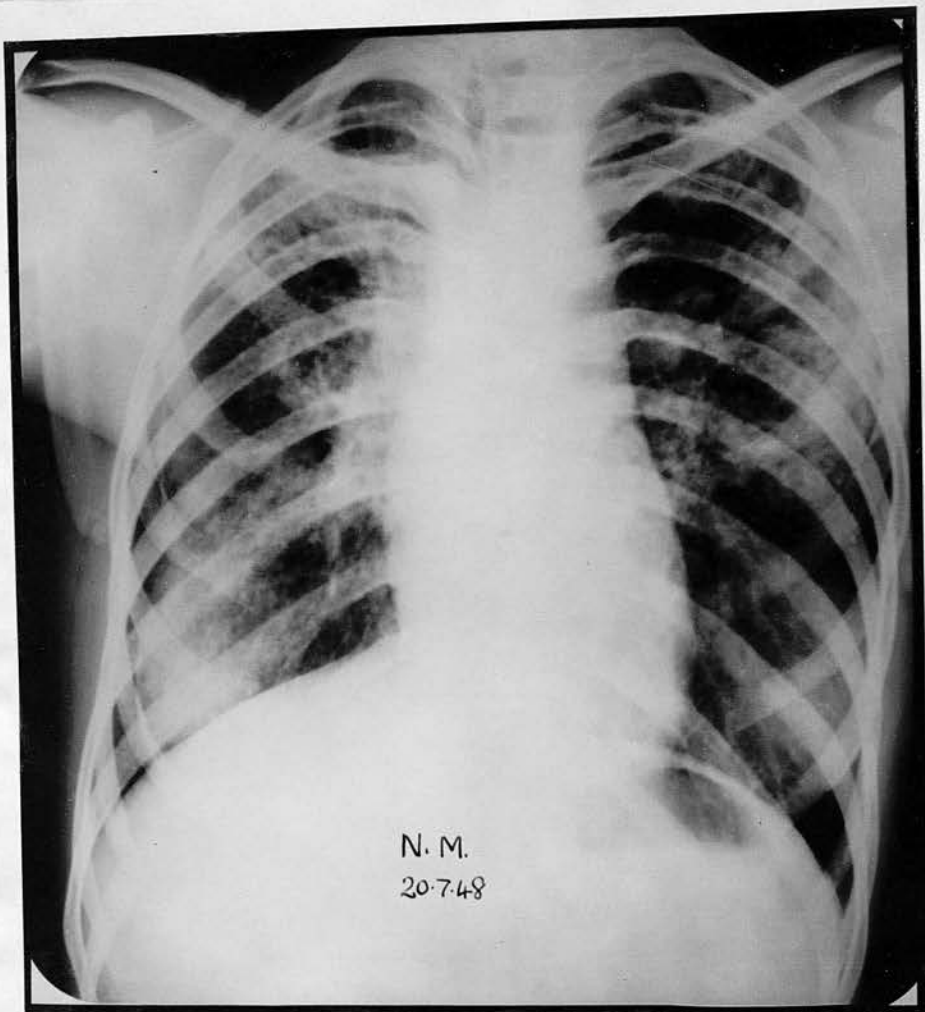


FIG. 13. N.M. Before treatment.

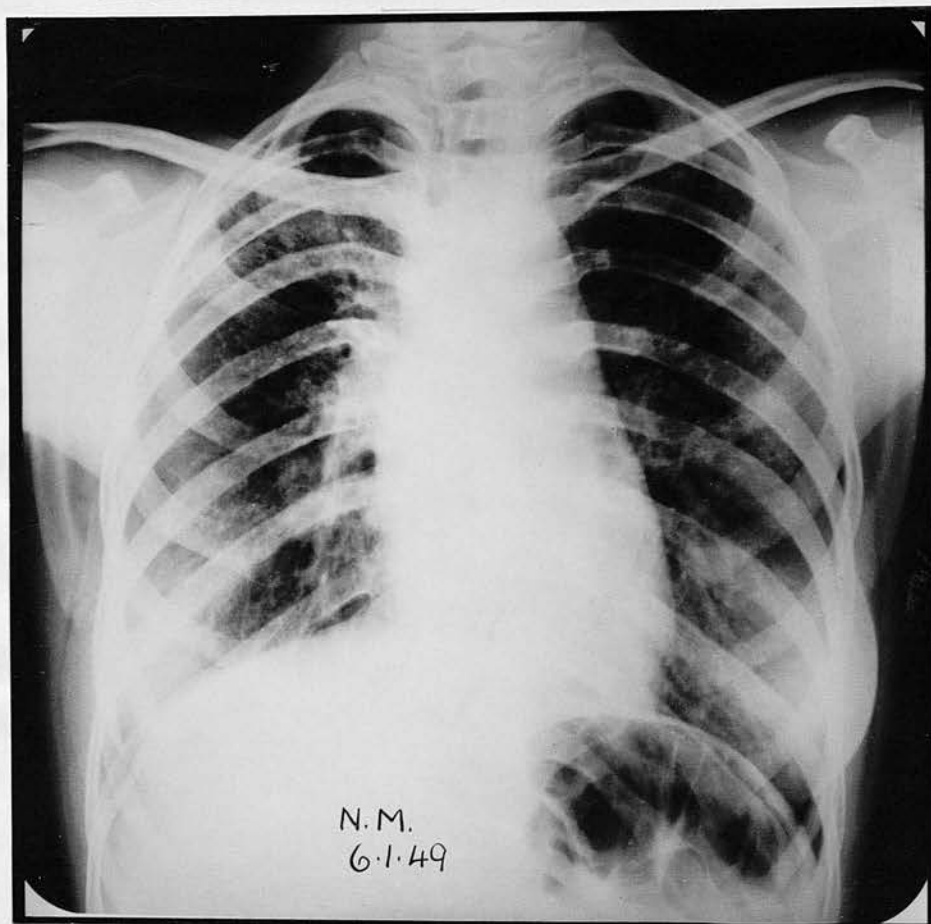


FIG. 14. N.M. After 12 weeks treatment.

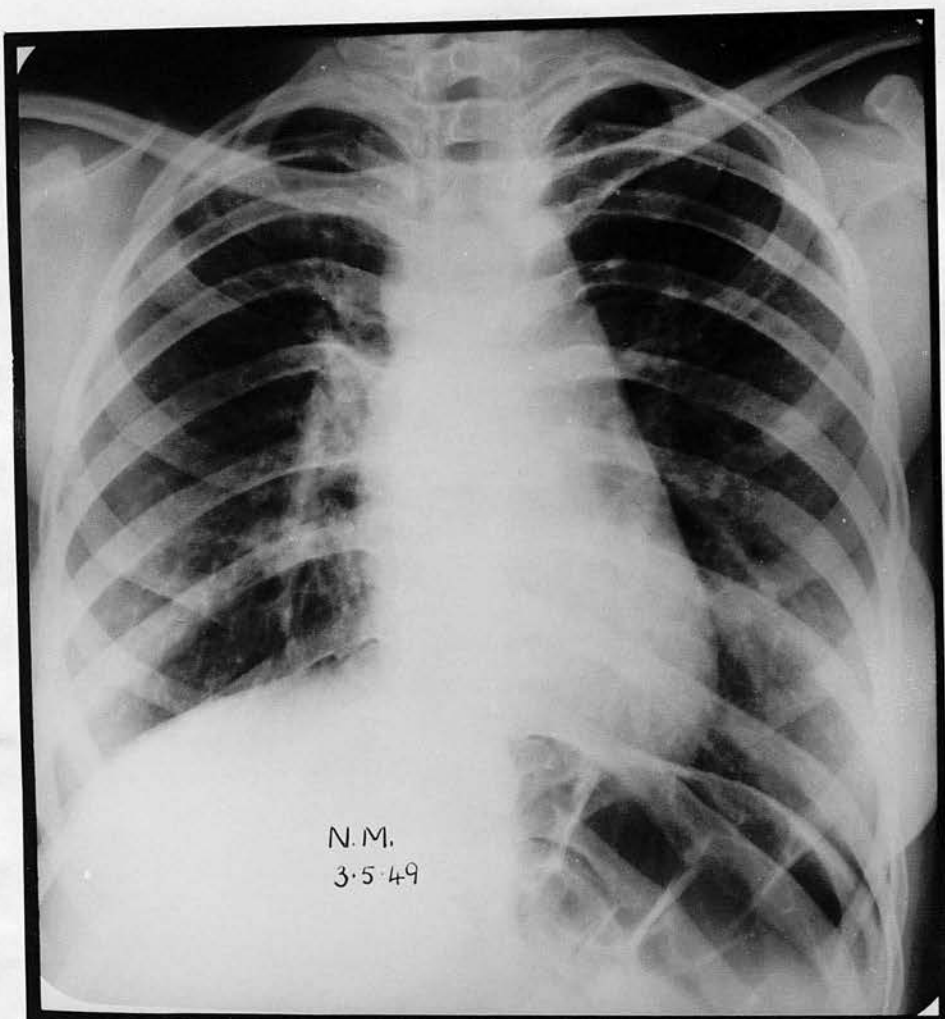


FIG. 15. N.M. After 24 weeks treatment.

Case 2. A.W. A young woman of 28 who gave a history of ill-health dating from a right sided pleurisy fifteen months previously. Until then her general health had been fairly satisfactory except that she had been liable to bronchitis with colds.

On admission the patient was pale and thin with a toxic appearance. Weight 7st. 5½lbs. The physical signs were those of cavitation below the left clavicle with moist accompaniments posteriorly in the interscapular region.

The X-ray picture (Fig.16) revealed bilateral pulmonary tuberculosis. Left lung - apical and upper zone mottling with infraclavicular cavity 3 cm. in diameter. Commencing cavitation medial to this cavity. Right lung - scattered mottling in apex, upper and mid-zones with confluence and commencing cavitation in relation to anterior end of 2nd rib. Costo-diaphragmatic sinus obscured.

Tubercle bacilli were abundantly present in the sputum.

#### Treatment

A left artificial pneumothorax was induced but this immediately provoked a marked pyrexial upset with the rapid development of a complicating effusion. The pneumothorax was therefore abandoned and treatment was limited to bed rest until the commencement of P.A.S. therapy two months later.

Again a course of 12 weeks was given followed by/

by a month's rest and a further 12 weeks of the drug.

Dosage: 15 gm. daily. A higher dose provoked signs of intolerance such as diarrhoea and vomiting and it was found that this dose represented the upper limit of the patient's tolerance to the drug.

Average blood level: 5.2 mgm. per cent.

#### Progress under treatment

In the period under consideration, a period of twenty-eight weeks, the patient's general condition improved slightly and a gain in weight of 6 lbs. over the period was recorded.

The B.S.R., after an initial fall, fluctuated, eventually returning to its original level.

The sputum was persistently positive on direct smear.

Initially a diminution in moisture occurred on physical examination and for a short period no moisture could be detected. This improvement was however only temporary and clinical activity recurred towards the end of the second course.

Radiological changes: Fig.17 gives the position radiologically at the commencement of the P.A.S. therapy. It will be seen that deterioration had taken place during the period of bed rest following the failed left pneumothorax (cf. Fig.16). Fig.18 represents the result of treatment. A degree of resolution of the individual foci in both lungs occurred with diminution in the size of the left/



left infraclavicular cavity. Original size 4 cm. x 3 cm.; size after treatment 2 cm. x 1.5 cm. The lesions also appeared to have undergone a certain amount of fibrotic change. It is worthy of note that the maximum improvement occurred in the first three months of treatment and no appreciable change took place in the last month.

#### Summary

General condition improved very slightly.

Some improvement in radiological appearances.

#### Conclusions

Despite the improvement, the lesions remain highly active and extensive. No other treatment is possible or indeed likely to produce benefit, and it is unlikely that continuance of P.A.S. will be effective in controlling the lesions further. The claim in this patient, therefore, is only that the drug converted a rapidly progressive lesion into a temporarily retrogressive one. No lasting benefit will accrue and the further management of the case is a difficult problem.

Prognosis: Not materially altered by treatment.

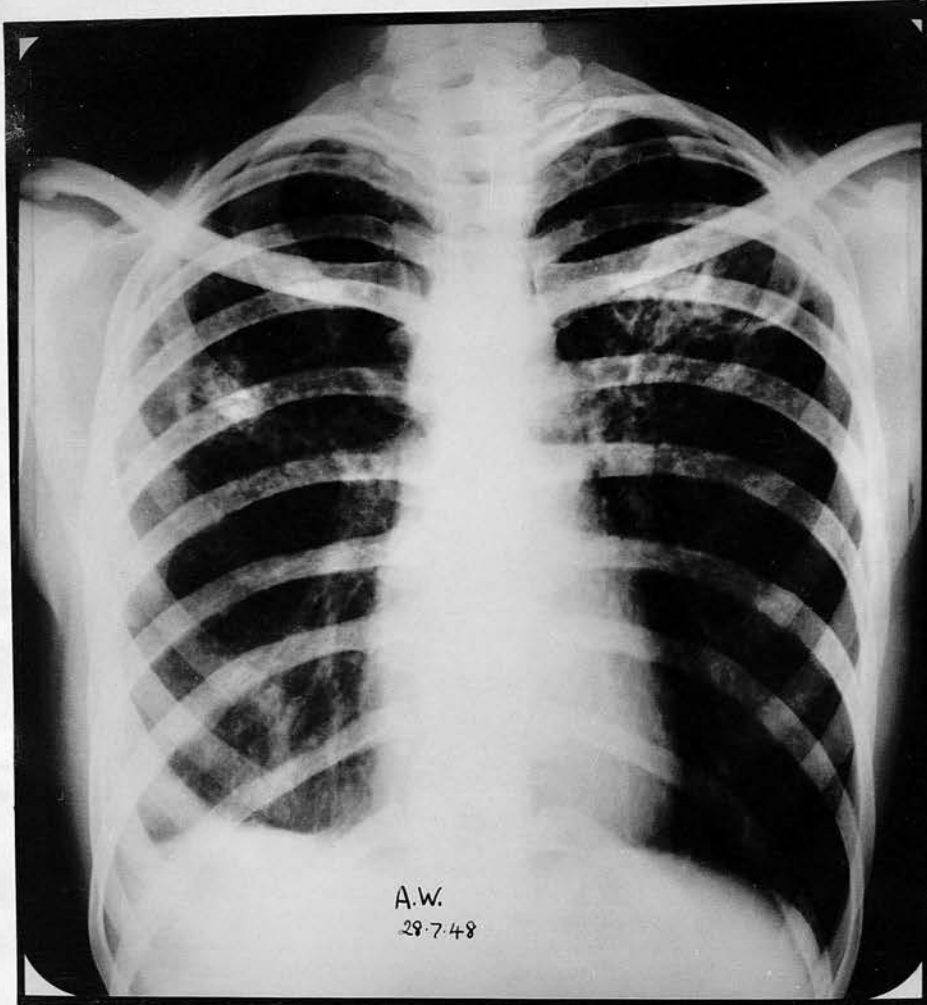


FIG. 16. A.W.

On admission

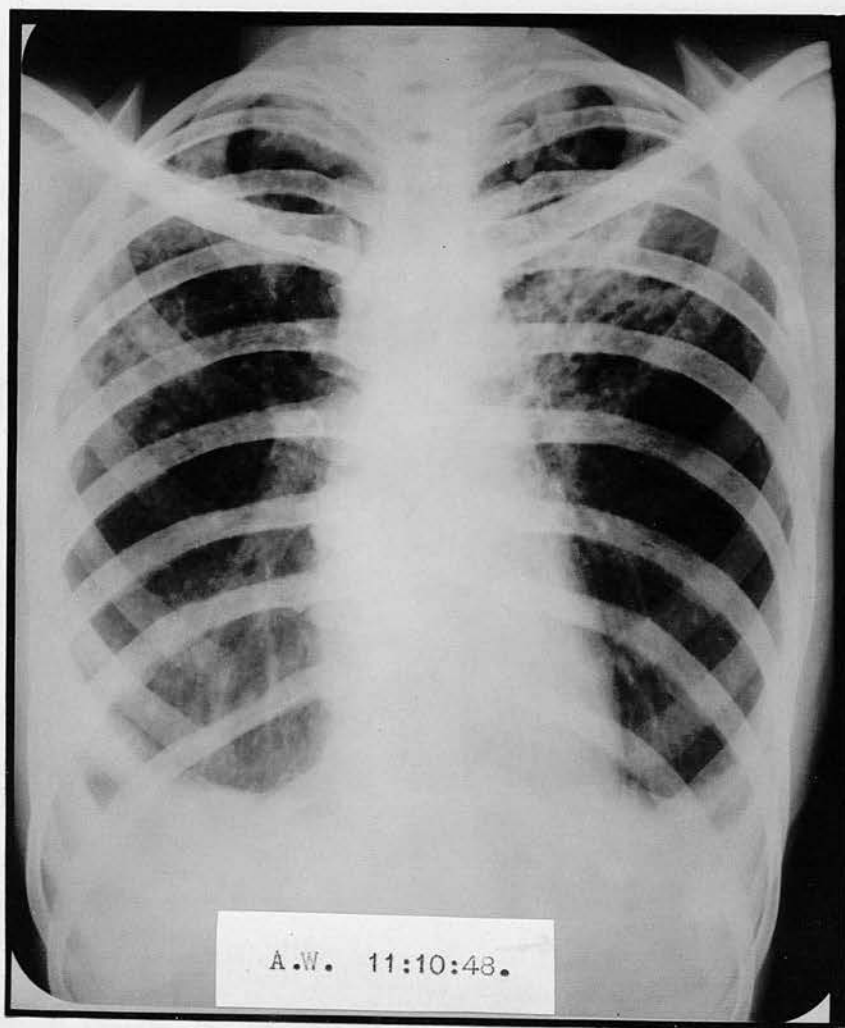


FIG. 17. A.W.

Before P.A.S.

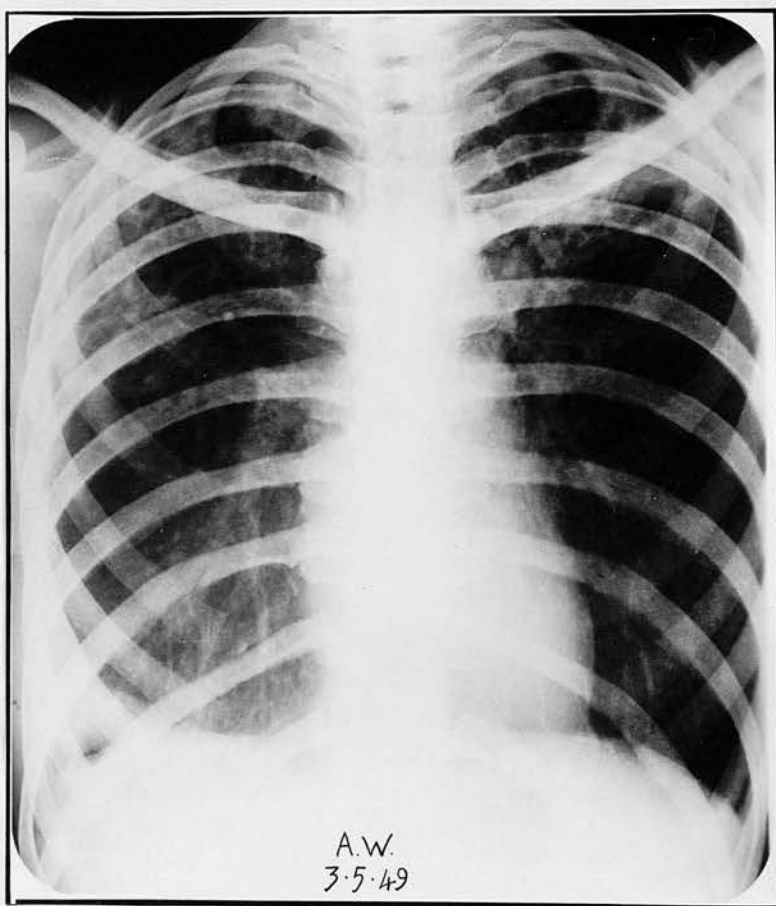


FIG. 18.   A.W.   After 24 weeks treatment.

Case 3. J.L., a young woman of 20 who dates her illness from a confinement five months previous to admission. Her main complaints were cough, pains in the chest and loss of weight. Nothing relevant was elicited in the past history.

When first seen she was thin with a high colour. Weight was 7st. 11lb. Physical signs in the chest consisted in impaired resonance over the right upper zone, with, on auscultation post tussic crepitations below both clavicles. The X-ray picture (Fig. 19) revealed bilateral pulmonary tuberculosis. Right lung: extensive mottling in upper zone with large cavity (14 cm. x 3.5 cm.) immediately below clavicle and smaller cavity inferior to first. Left lung: Scattered mottling throughout upper and mid-zones, densest at the periphery. No obvious excavation.

Tubercle bacilli were abundantly present in the sputum.

#### Treatment

The disease was considered too extensive and active for any form of collapse therapy. A short period of bed rest alone occurred before the commencement of P.A.S. therapy. Again two twelve week courses of the drug were given with a month's rest between.

Dose: 15 gm. daily in the first course. Attempts to give a higher dosage were followed by sickness/

sickness and diarrhoea. At the beginning of the second course, at the patient's request, the dose was stepped up to 25 gm., and at this time she was able to tolerate it with only occasional sickness.

Average blood level: 4.8 mgm. per cent.

### Progress

During the first course of treatment a gradual increase of weight was recorded and in the rest period this improvement was maintained. During the second course however gradual weight loss took place. This secondary deterioration was also reflected in the general condition and in the B.S.R. readings which, following a marked initial fall, gradually rose, although not quite to the original level

**Bacteriology:** At the end of the first course tubercle bacilli could only be isolated from the gastric washings but towards the end of the second course sputum, in which bacilli were present on direct smear, reappeared.

**Radiological appearances:** During the first three months of treatment resolution and contraction of individual foci occurred in both lungs. Diminution in the size of the right infraclavicular cavity also took place (4 cm. x 3.5 cm. to 3.5 cm. x 2.5 cm.). During the second course resolution continued to a certain extent but not as markedly. Increase in the size of the cavity in the right lung occurred, however, with coalescence of the two previously present, resulting in a cavity, 5 cm. x 4 cm., appreciably/



appreciably larger than the original. (Figs. 20 and 21)

### Summary

Initial and promising improvement in all findings not maintained despite the continuance of the drug.

### Conclusion

The original object in the use of P.A.S. in this patient was to promote healing in the infiltrative disease in the left lung with the hope that thoracoplasty would eventually become possible to control and close the cavity in the right lung. This was not achieved; no further benefit can be expected from the drug; the disease is active and progressive and again the future management of the patient presents a serious problem. Deterioration may have been retarded but it has not been halted.

Prognosis: Unaltered by treatment.

Addendum: Since the cessation of treatment deterioration has continued.

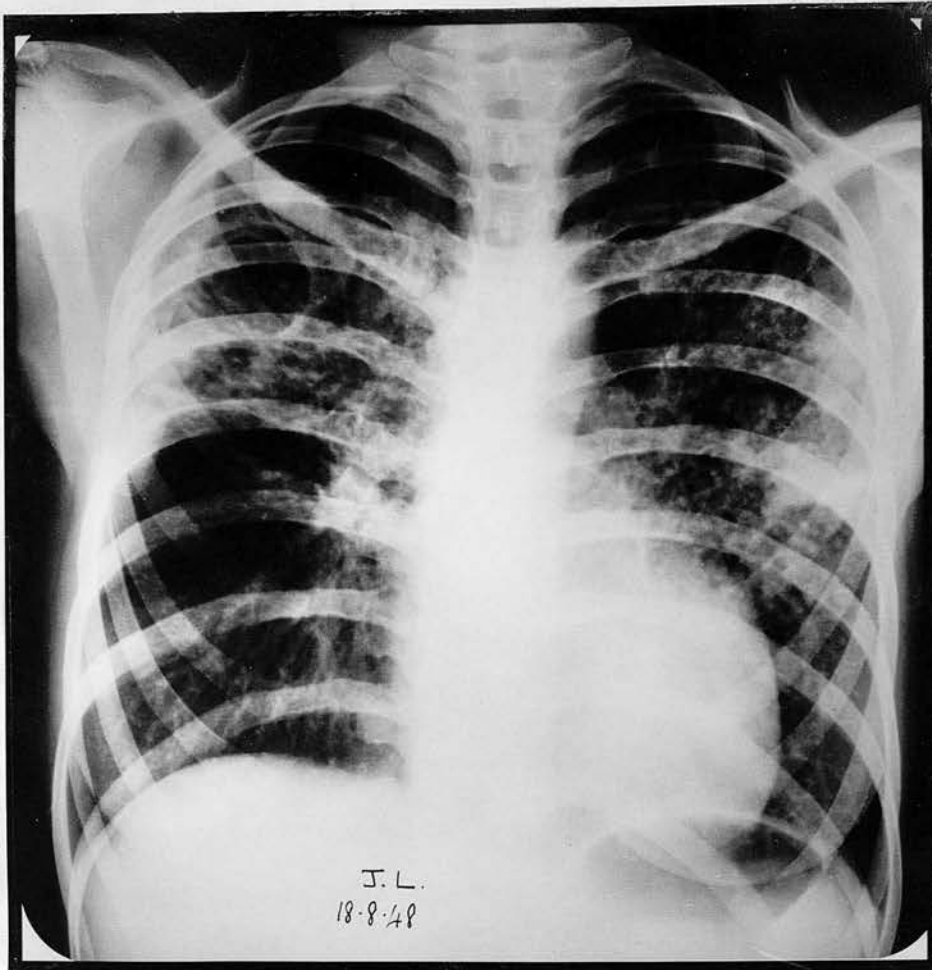


FIG.19. J.L.  
Before P.A.S.



FIG. 20. J.L.  
After 12 weeks  
treatment.

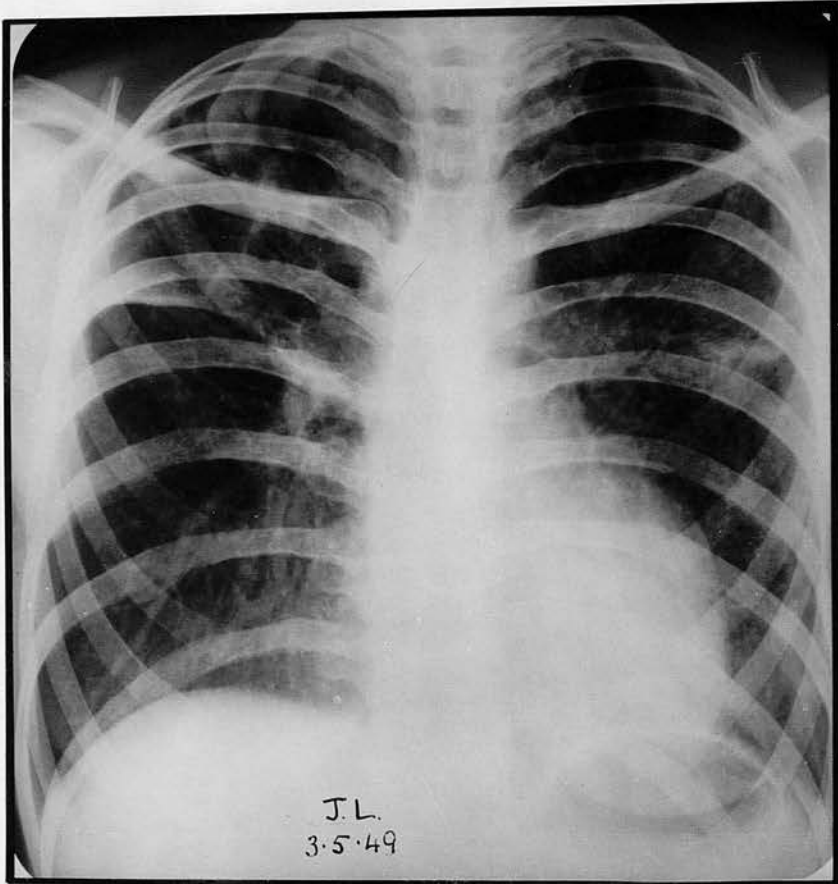


FIG.21. J.L. After 24 weeks treatment.

Case 4. A.P. A married woman of 29 who was discovered to be the subject of pulmonary tuberculosis in the later months of pregnancy. Bilateral pleurisy had occurred previously, one in relation to a pregnancy 2 years before, and the other five months before admission. The disease although not extensive was bilateral and active and this together with the pregnancy presented a difficult problem in management.

When first seen her general condition was good, clinical activity could not be detected but the radiological findings were as follows:

Right lung - discrete mottling in the apex and below the clavicle without excavation. Left lung - mottling at the apex and in the upper and mid-zones, fairly dense at the periphery of the upper and mid-zones. No excavation.

Tubercle bacilli were isolated from the gastric washings.

#### Treatment

The pregnancy was allowed to continue to term, when delivery was by Caesarian section. Immediately on return from the Maternity Hospital a course of P.A.S. was started.

Length of course: 12 weeks

Dosage: 25 gm. daily

Average blood level: 5.2 mgm. per cent

Signs of intolerance: nil.

Progress/

### Progress

During treatment her general condition improved, 5 lbs. in weight was gained and the B.S.R. reading fell from 43 to 5.

After treatment no growth was obtained from culture of the gastric washings.

Radiological changes: The X-ray picture at the cessation of treatment showed consolidation and contraction in the lesion behind the <sup>right</sup> clavicle with some resolution in the small infiltration below the clavicle. The changes in the left lung infiltration were not so marked although a degree of resolution had taken place. On radiological appearances alone the lesions could not be definitely stated to be quiescent.

### Summary

Improvement in general condition.

Improvement in X-ray appearances.

### Conclusions

The expected course of this patient's disease must be a matter for conjecture and, although it is realised that the extent of the disease itself did not constitute a serious danger, the presence of such lesions in a pregnant woman where they cannot be brought under control before parturition necessitates careful consideration of the problem. It is impossible to determine how much of the resultant improvement was due to the bed rest imposed in the puerperal period and how much to the administration/



tration of the drug. But it is universally recognised that the puerperal period is the danger period for the tuberculous woman and that this time was passed without deterioration is worthy of note.

#### Prognosis

Probably improved as a result of P.A.S. therapy.  
Addendum: This patient seven months after treatment, continues well and has been able to resume duties at home.

Case 5. F.C. A young woman of 23 whose disease had been discovered when she was X-rayed as a contact of a sister who had died of pulmonary tuberculosis and of another who was receiving treatment in the Sanatorium. The disease when she was first seen was minimal in extent but tomography had revealed the presence of a small cavity, 1 cm. in diameter, in both lungs. After several months of pneumoperitoneum and a right artificial pneumothorax tubercle bacilli were found on direct smear in the sputum for the first time. The collapse measures appeared mechanically efficient and therefore, at this juncture, it was decided to give this patient a course of P.A.S.

Length of course: 12 weeks

Dosage: 25 gm. daily for  $2\frac{1}{2}$  weeks. This resulted in a considerable amount of nausea and sickness and the dose was reduced to 15 gm. daily. This dosage was tolerated well.

Average blood level: 4 mgm. per cent.

### Progress

As this patient had already been under treatment for eight months before the course of P.A.S. began, little further improvement in her general condition etc., was to be expected. 3 lbs. weight were lost during the course. The B.S.R. was also already within normal limits at the start of the course.

Following treatment with P.A.S. culture of the sputum/

sputum was negative for tubercle bacilli.

Radiological changes: The small cavities present in both lungs at the start of the P.A.S. course were proved by tomography to be closed following treatment.

#### Conclusions

Conversion of the sputum and closure of small cavities took place during the period of P.A.S. therapy but whether this was attributable to the continuance of the collapse therapy or to the P.A.S. it is impossible to say.

#### Prognosis

This was never grave and cannot therefore be said to have been appreciably altered by the course of P.A.S.

Addendum: Cavities remain closed and sputum negative on culture 8 months after cessation of P.A.S. treatment.

Case 6. A.J. A young girl, aged 20, of Malayan nationality who had travelled to this country with her British husband after insufficient treatment had been given for a tuberculous lesion in the left lung. When first seen bilateral active tuberculosis was present and although the disease was not extensive a small cavity was present in the right lung. Fig. 22 gives the position when the patient came under treatment; the cavity in the right lung appears to have closed spontaneously but the disease in the left lung behind and below the clavicle is active and was slightly more extensive than in the original picture. In view of the fact that this lung had previously been treated by artificial pneumothorax, and also because of the patient's nationality with, therefore, the possibility of a poor native resistance, it was decided to supplement bed rest with P.A.S.

Tubercle bacilli were grown on culture of the sputum.

#### Treatment

Length of course: 12 weeks

Dosage: 25 gm. daily

Average blood level: 6 mgm. per cent.

Signs of intolerance: nil.

#### Progress

Improvement in the general condition was marked but this was to be expected with simple measures alone as the patient's home circumstances were poor. Gain/

Gain in weight amounted to  $3\frac{1}{2}$  lbs. Fall in B.S.R. reading from 29 to 3. Following treatment no growth was obtained on culture of the gastric washings.

Radiological changes: Fig. 23 gives the X-ray appearances following the cessation of treatment. Contraction and hardening of the right infraclavicular lesion has occurred. Some resolution and fibrosis have taken place in the infiltration in the left lung.

#### Summary

Improvement in general condition.

Improvement in radiological appearances.

#### Conclusions

It cannot be certainly stated in this case that the above improvement would not have taken place if bed rest had been the sole therapeutic measure. Nevertheless in view of the patient's nationality deterioration in the lesions might have been expected so that it could be claimed that P.A.S. struck the balance in favour of improvement.

#### Prognosis

Never serious, possibly slightly improved.

Addendum: The patient remains well eight months after cessation of treatment and has resumed home duties.



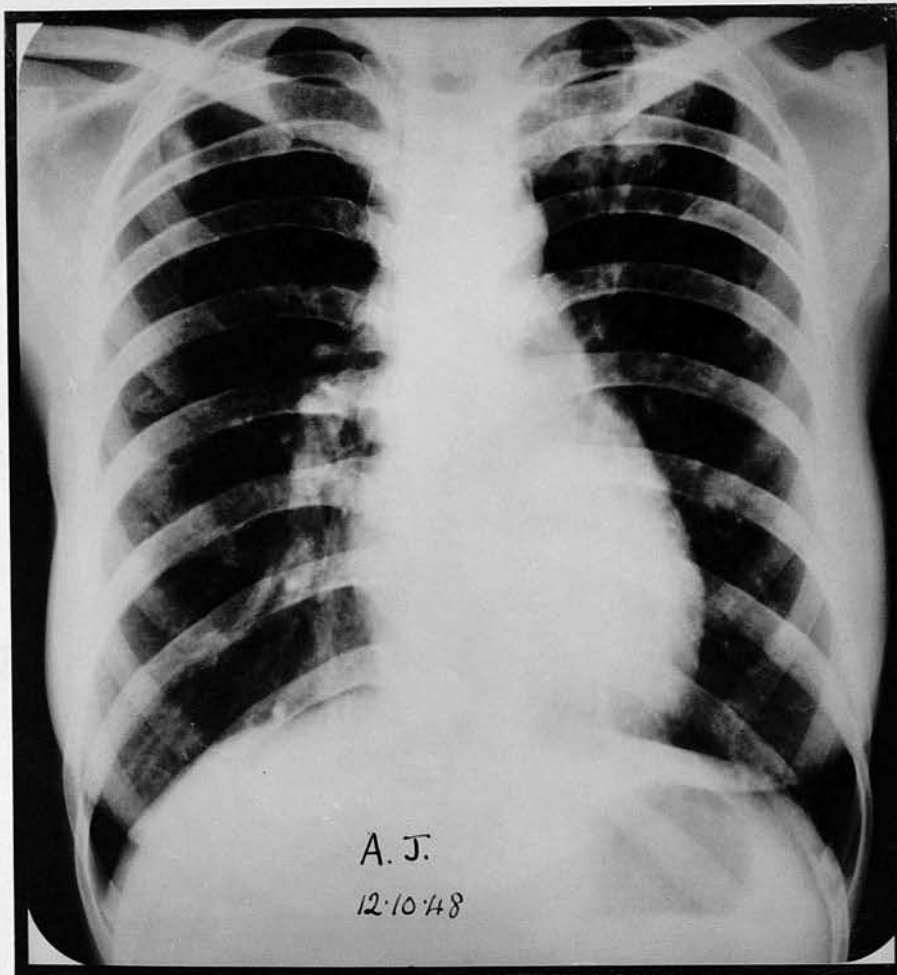


FIG. 22. A.J. Before treatment.



FIG. 23. A.J. After 12 weeks treatment.

Case 7. A.C. A young girl, aged 16, who is included in the group as an example of complete intolerance to the drug. Because this necessitated the cessation of the course after only 39 gm. had been given she has not been included in any table of results presented.

Although the history was short, 2 months cough, the right lung was extensively involved and a recent dissemination had taken place in the left lung when the patient first came under observation. Marked toxæmia with a swinging temperature (range  $98^{\circ}$ - $103^{\circ}$ ) was a feature of the case. Active measures were contraindicated for this reason and it was hoped that chemotherapy would reduce the activity of the lesions sufficiently to permit some form of collapse therapy later.

The recommended dose of P.A.S. excited violent sickness almost immediately. Reduction of the dose was not sufficient to abolish the vomiting and as this vomiting was quickly rendering the patient's condition critical the drug was discontinued completely. The intestinal upset subsequently subsided gradually but steady deterioration took place in the local and general condition and the patient died six months later.

Case 8. T.G. A married woman of 31 who dates her ill-health again to a confinement one year before admission. The principle symptoms had been cough and loss of weight. A disturbing feature of the case was that two other members of her family, her father and brother, were suffering from advanced tuberculosis.

When she was first seen the disease which was of the active infiltrative type was mainly confined to the left lung. A fine seeding only was present on the right side. The left lung was treated by artificial pneumothorax but this unfortunately was complicated by a chronic pleural effusion which resulted in an obliterative pleuritis. A unhealed cavity remained at the apex. During this time the seeding in the right lung had gradually become more extensive. Thoracoplasty was the only remaining method of treatment that was likely to prove successful in obliterating the lesion at the left apex but this could not be considered with active disease in the right lung. With the hope, therefore, that P.A.S. might bring the patient within the scope of surgical collapse, especially since the seeding in the right lung was of the fine nature which might be expected to respond to chemotherapy, it was decided to give this patient a course of P.A.S.

The sputum at this time contained numerous tubercle bacilli on direct smear.

Length of course: 12 weeks

Dosage/

Dosage: 25 gm. daily for 4 weeks but sickness and diarrhoea became troublesome at this time and the dose was cut to 15 gm. daily, at which level it was maintained for the remainder of the course.

Average blood level: 3.3 mgm. per cent.

### Progress

Again a considerable period of treatment had taken place before the course of P.A.S. began so that dramatic changes in the general condition etc., were not to be expected. Much of the systemic upset had been eliminated before treatment began. A slight loss in weight (3 lbs.) occurred.

Following treatment no growth was obtained on culture of the sputum and repeated direct smears were negative.

Radiological changes: Figs. 24 & 25 illustrate the radiological appearances before and after treatment. It will be seen that some resolution has occurred in the fine seeding in the right lung. No appreciable change has taken place in the left lung.

On careful consideration the improvement in the right lung was not deemed of sufficient degree to justify the hope that a second course of the drug would produce further benefit.

### Summary

Resolution of certain fine foci in right lung.

### Conclusions

There can be little doubt that the resolution which/

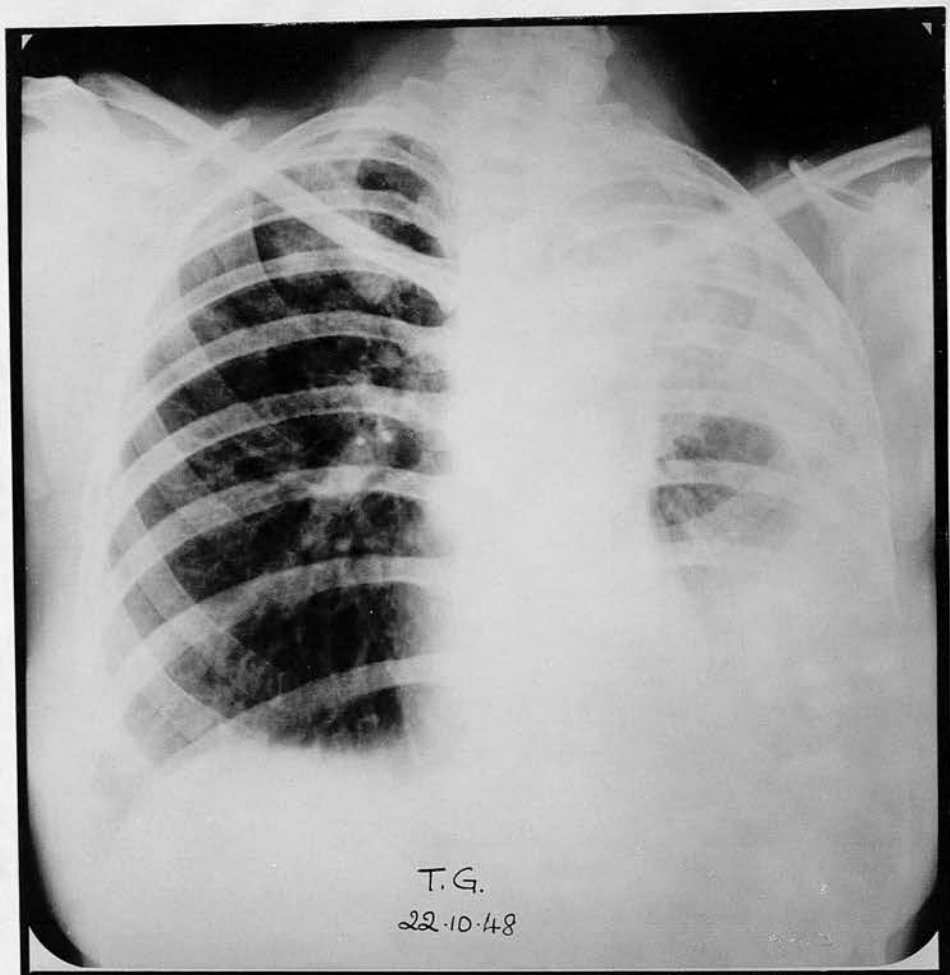


FIG. 24. T.G. Before P.A.S. therapy.

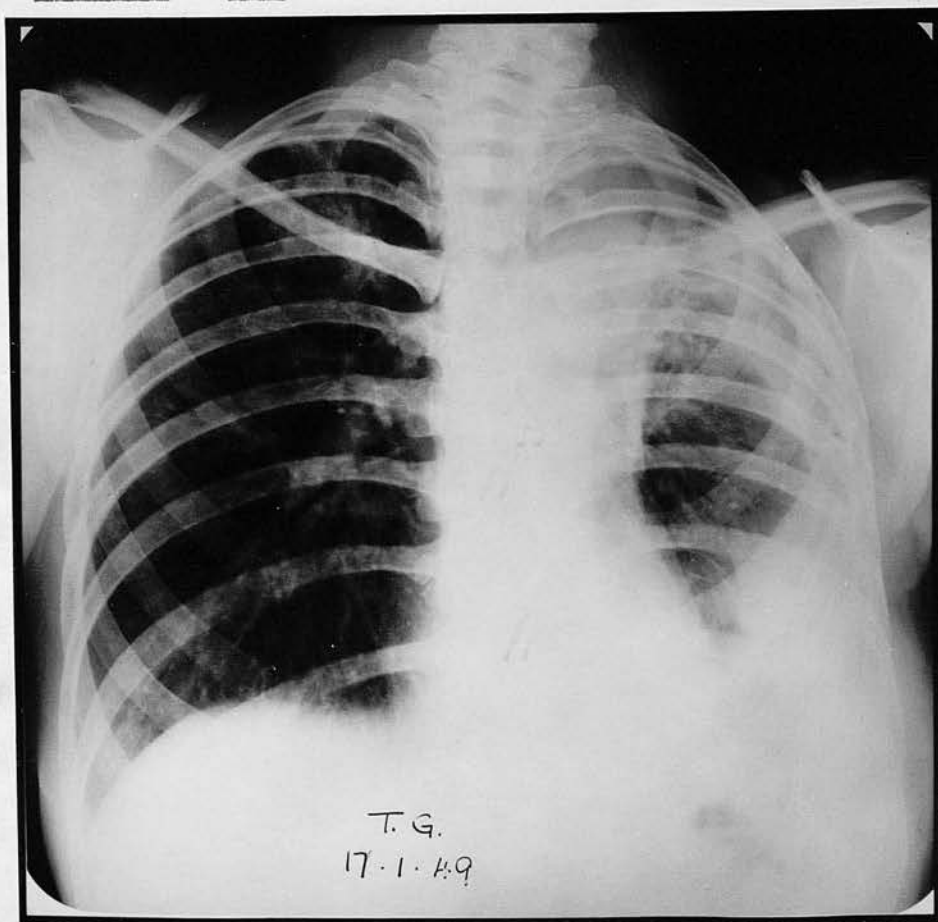


FIG. 25. T.G. After treatment. ( 12 weeks).



Case 9. D. McM. This patient, a man aged 43, is one in whom the presenting symptom was haemoptysis and the case is interesting in that it presents an opportunity for the study of the effect of the drug on lesions whose age is radiologically proved to be no more than 12 days. When the first picture was taken (Fig. 26) an active tuberculous infiltration was present below the left clavicle. The lesion was just beginning to excavate. 12 days later (Fig. 27) a widespread, bronchogenic dissemination had occurred, all zones of the left lung being involved with also a contralateral spread involving the upper and mid-zones of the right lung.

Tubercle bacilli were abundantly present in the sputum.

#### Treatment

It was immediately decided to give this patient treatment with P.A.S. In the first instance a course of twelve weeks was given. The result was disappointing since, following some initial improvement, deterioration took place both in his general condition and in the radiological appearances (Fig. 28).

At this stage streptomycin became available for the treatment of tuberculous broncho-pneumonic lesions and a course of this drug was started. Two months later (Fig. 29) no appreciable change was detectable in the X-ray appearances except slight contraction of the lesion in the right lung. There/

There was at this time very slight improvement in his general condition.

On account of this it was decided to continue with the streptomycin (dosage 0.5 gm. twice daily) but to combine it now with a 2nd course of P.A.S. The dosage during this course was 18 gm. daily which was tolerated well. One month later, for the first time, an appreciable improvement was noted in the X-ray picture. (Fig. 30 ).

Fig. 31 illustrates the radiological picture on completion of the second 12 weeks course of P.A.S., during which time the course of streptomycin, which had lasted four months, finished.

Average blood level: 4.5 mgm. per cent.

### Progress

Fig. 32 is a graphic representation of this patient's progress under treatment and summarizes conveniently the long and complicated picture which is entailed.

A point worthy of note, in addition, is the fact that on cessation of the first course of P.A.S., despite deterioration in all findings, tubercle bacilli which were previously abundantly present in the sputum were not demonstrable on direct smear although they were grown on culture. Following the combined course no growth was obtained on culture of the sputum.

Radiological changes: When response to treatment eventually became manifest the changes in the X-ray/

X-ray appearances consisted in resolution of the individual foci, the most striking feature; hardening of remaining foci, and cicatrization especially marked in the infiltration in the right lung.

#### Summary

Deterioration during first course of P.A.S.

No. change after two months treatment with streptomycin.

Striking improvement when streptomycin was combined with a second course of P.A.S.

#### Conclusions

There can be little doubt that P.A.S. alone and, possibly too, streptomycin alone were ineffective in controlling the recent infiltrative, widespread lesions in this patient. The improvement resultant upon the combination of streptomycin and P.A.S. was manifest rapidly and strikingly. Although the results in one such case are insufficient upon which to base conclusions it indicates a possible line of approach to this grave type of disease.

#### Prognosis

Considerably improved by treatment.

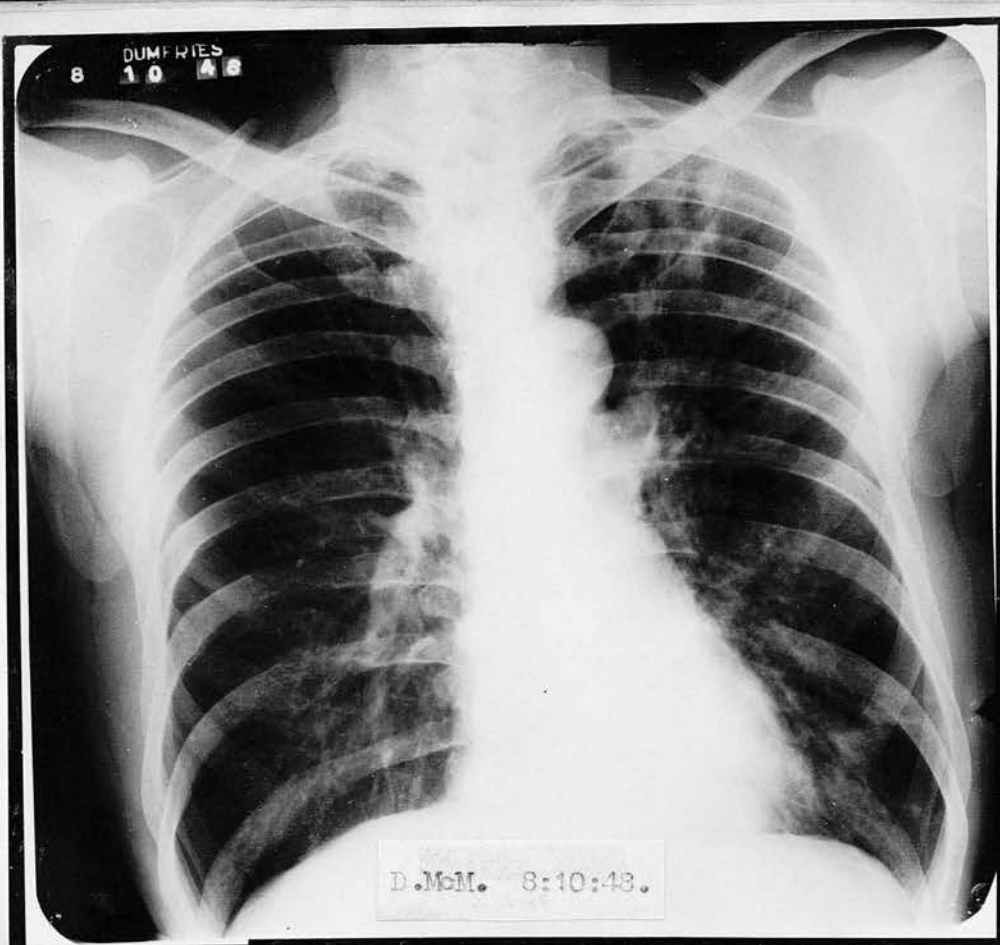


FIG. 26. D.McM. Before haemoptysis.

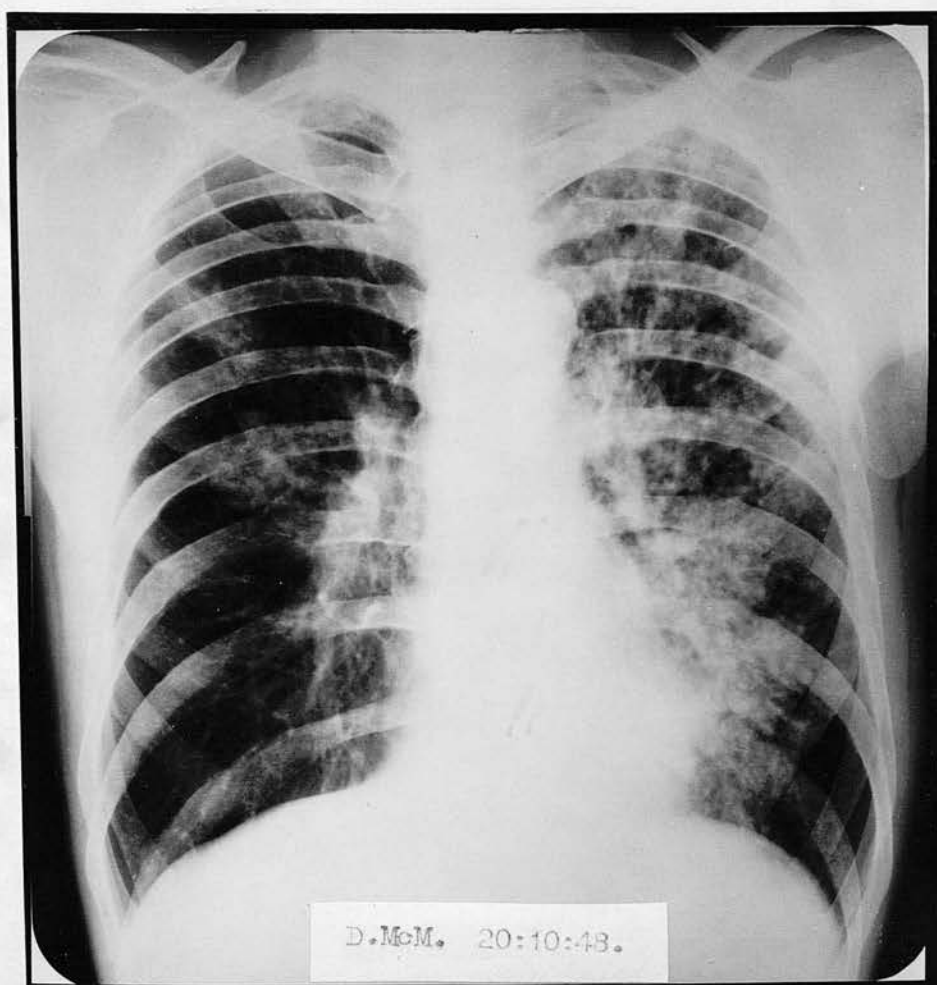
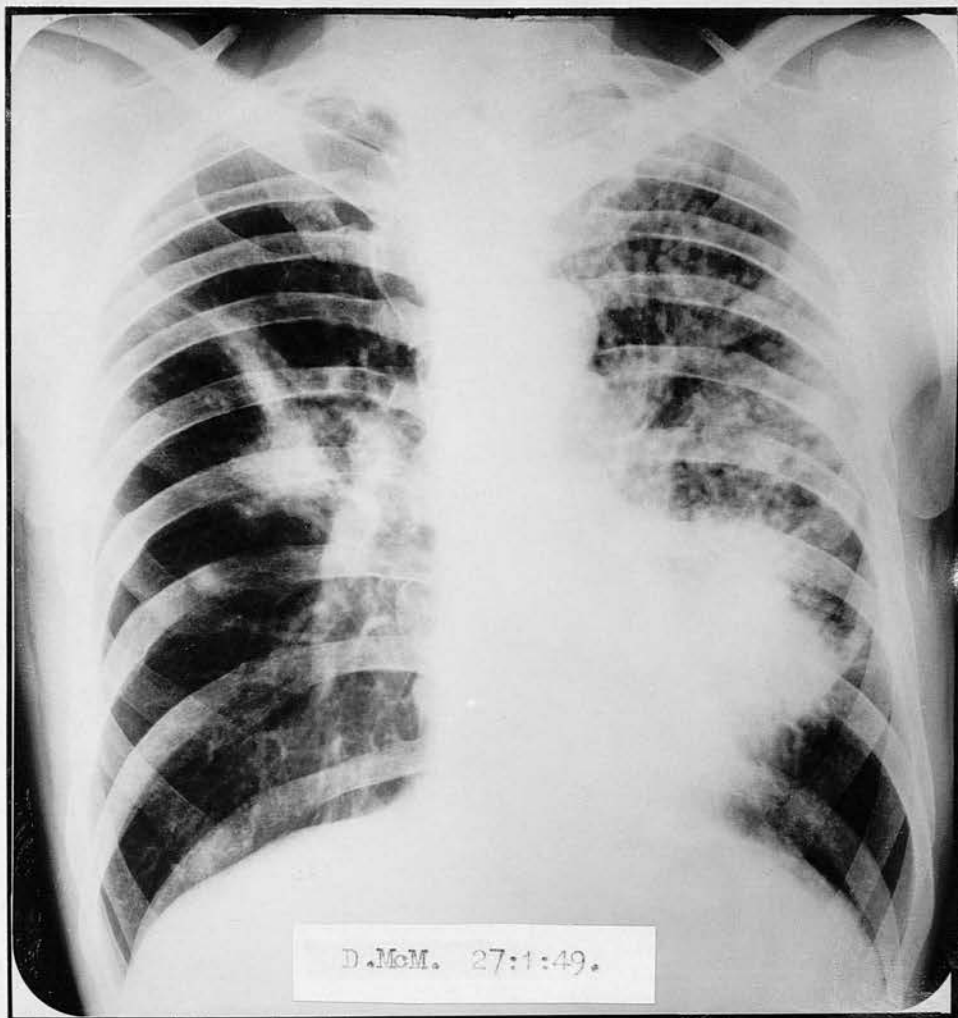


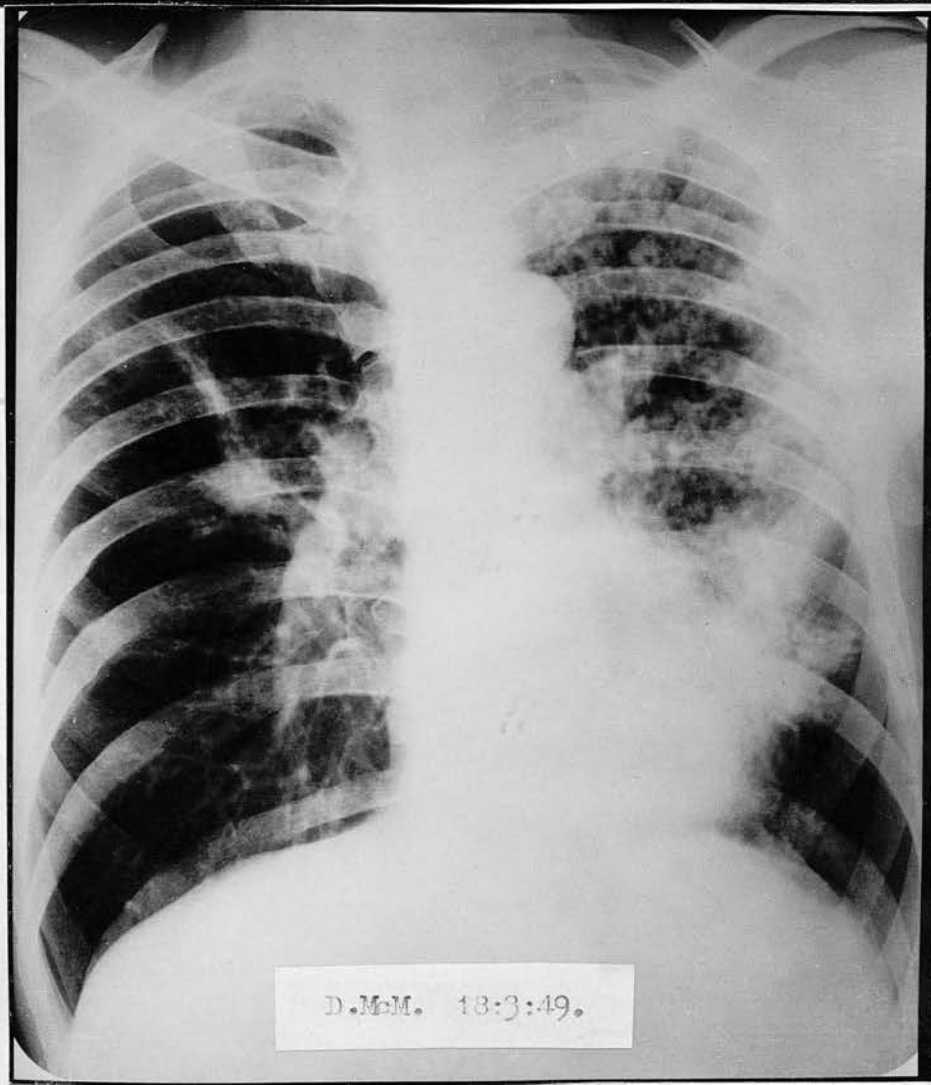
FIG. 27. D.McM. After haemoptysis, before P.A.S.





D.McM. 27:1:49.

FIG. 28. D.McM.  
After 12 weeks  
P.A.S.



D.McM. 18:3:49.

FIG. 29. D.McM.  
2 months later  
after strepto-  
mycin.



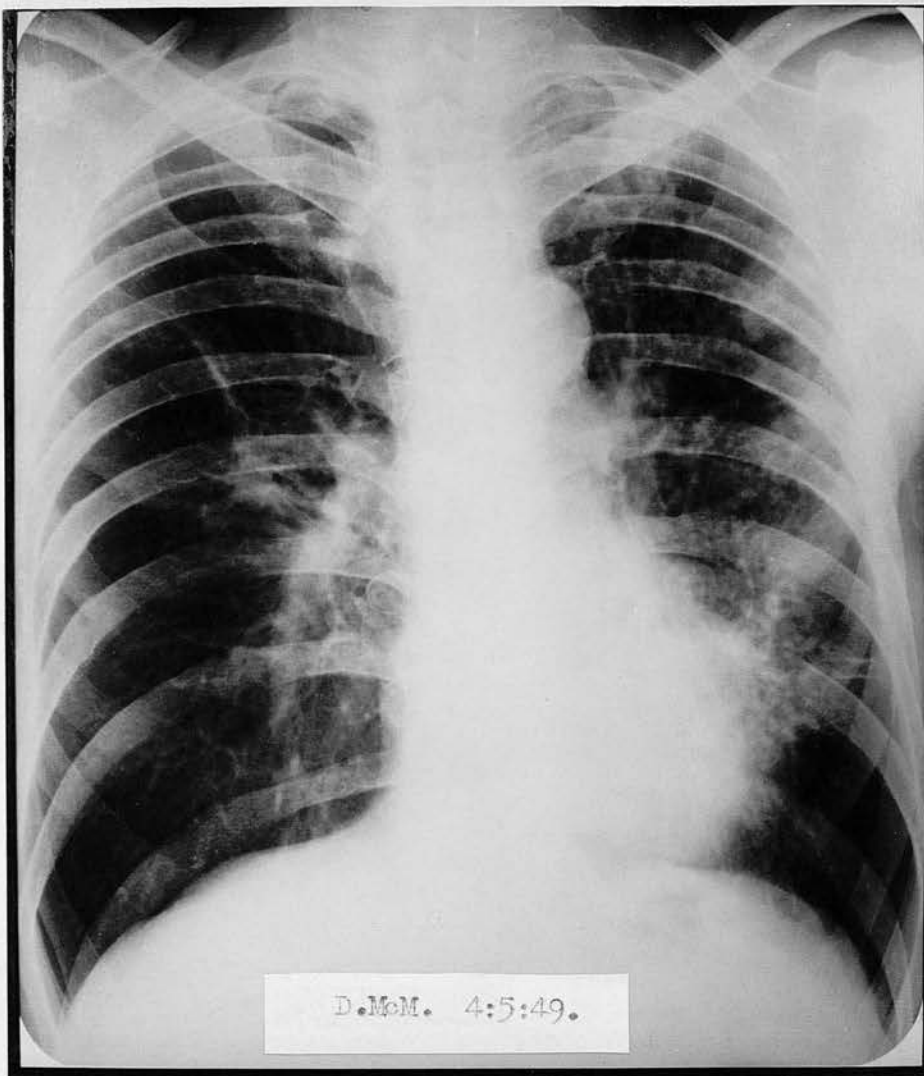


FIG. 30. D.McM.  
After 1 month  
combined P.A.S.  
& streptomycin.

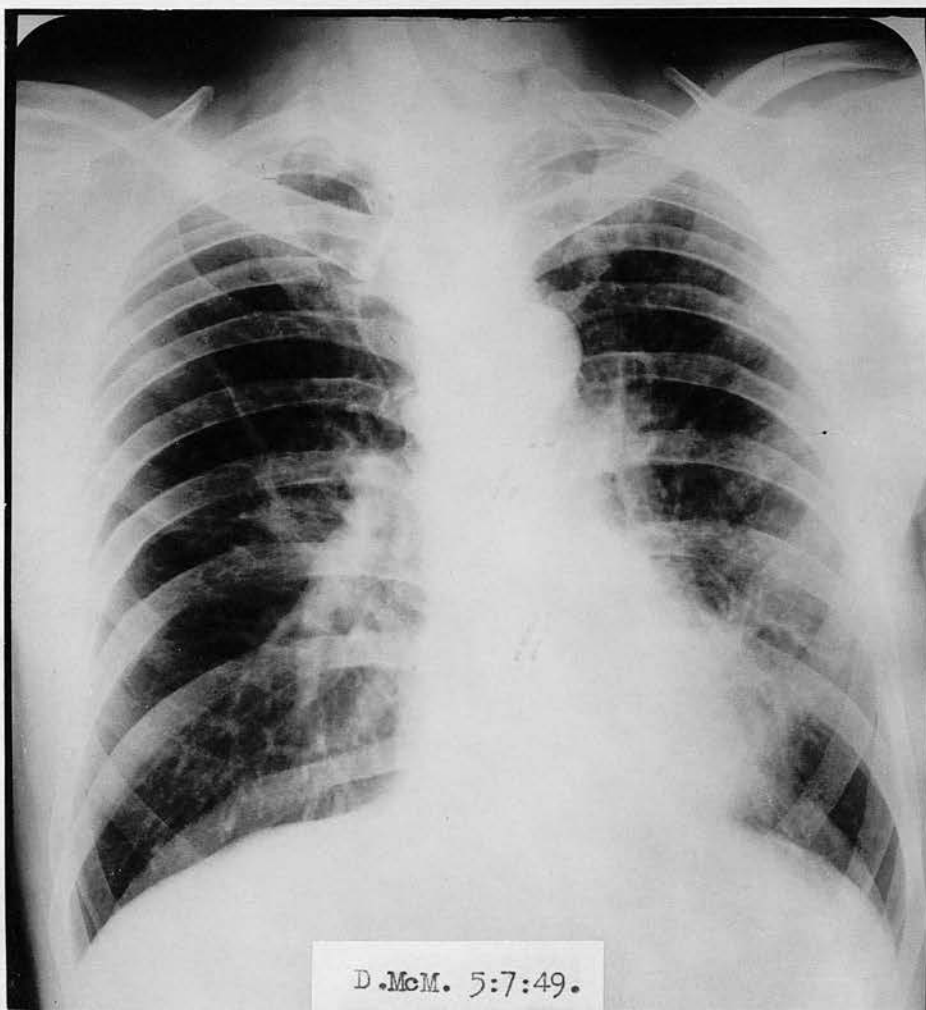


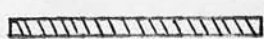
FIG. 31. D.McM.  
At completion  
of treatment.

LEGEND FOR FIG. 32.

D.McM. Progress under Treatment.

Average weekly temperature : average of morning  
and evening temperatures for each week.

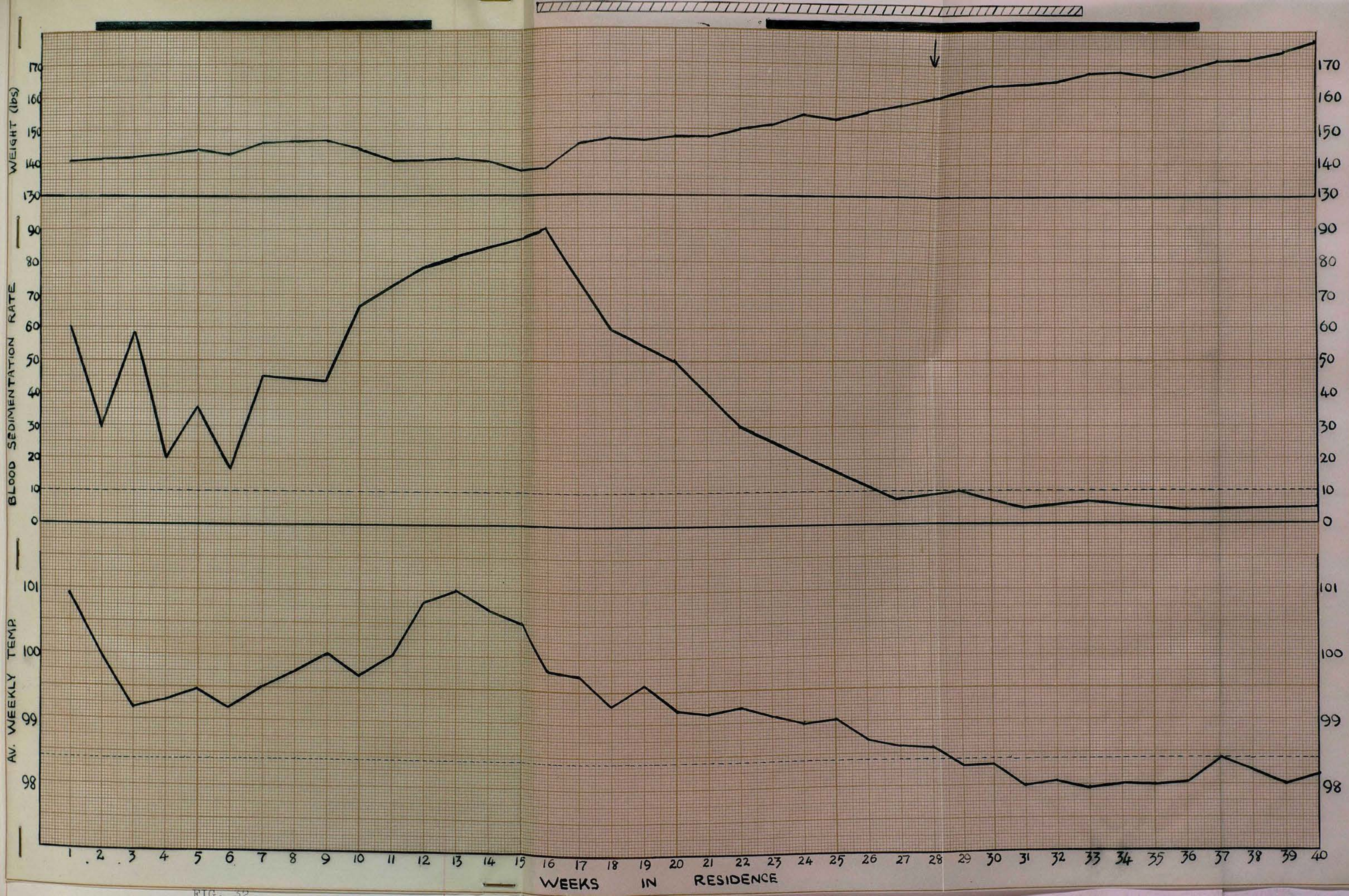
 P.A.S.

 Streptomycin.



Appreciable improvement in radiol-  
ogical appearances first demonstrated.







Case 10. R.W. A young man of 20 whose history of cough dated for only a month when an X-ray revealed bilateral pulmonary tuberculosis with cavity formation below the right clavicle. (Fig.33 ). He was pale and thin with a highly nervous temperament.

Tubercle bacilli were present in large numbers in the sputum.

#### Treatment

A right artificial pneumothorax was induced but, as might be expected from the X-ray appearances, the lung was broadly adherent over the apex and this treatment was abandoned. (Fig.34 ).

It was then decided to supplement bed rest with a course of P.A.S.

Length of course: 22 weeks with gap of two weeks at the end of 12th week.

Dosage: 25 gm. daily

Signs of intolerance: this patient was occasionally subject to diarrhoea and vomiting and, as he found the evening dose most irritating this was dispensed with. The daily total was maintained by increasing each of the remaining doses to 4 gm. and retaining the 5 gm. morning dose.

Average blood level: 6.3 mgm. per cent.

#### Progress under treatment

A notable feature in this case was the improvement in the general condition and mental attitude of a patient previously apprehensive and with a fear of the needle.

A slight loss in weight (2 lbs.) was recorded during treatment. The B.S.R. readings fell from 23 to 4. At the close of treatment culture of the sputum was negative for tubercle bacilli.

Radiological changes: Closure of the right infraclavicular cavity which occurred during treatment was proved by tomography. Partial resolution and fibrosis of the lesions particularly in the left lung were also recorded. (Fig. 35).

#### Summary

Improvement in general condition

Improvement in radiological appearances.

#### Conclusions

Again it cannot be certainly stated how much of the improvement recorded was attributable to bed rest alone and how much to the drug. Bilateral active disease with cavitation is always a serious problem and it is considered that part at least of the improvement must be attributable to the drug.

#### Prognosis

Improved as a result of treatment.

Addendum: 4 months after treatment his condition remains satisfactory.



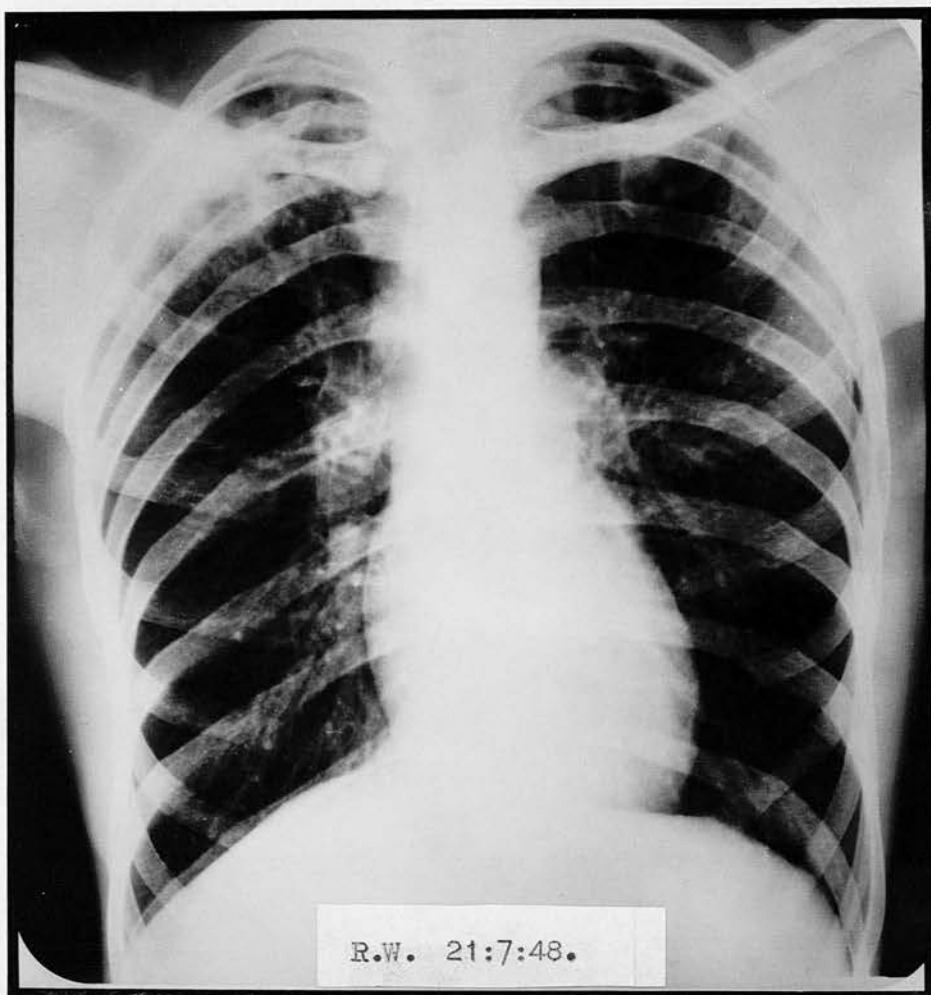


FIG. 33.   R.W.   On admission.

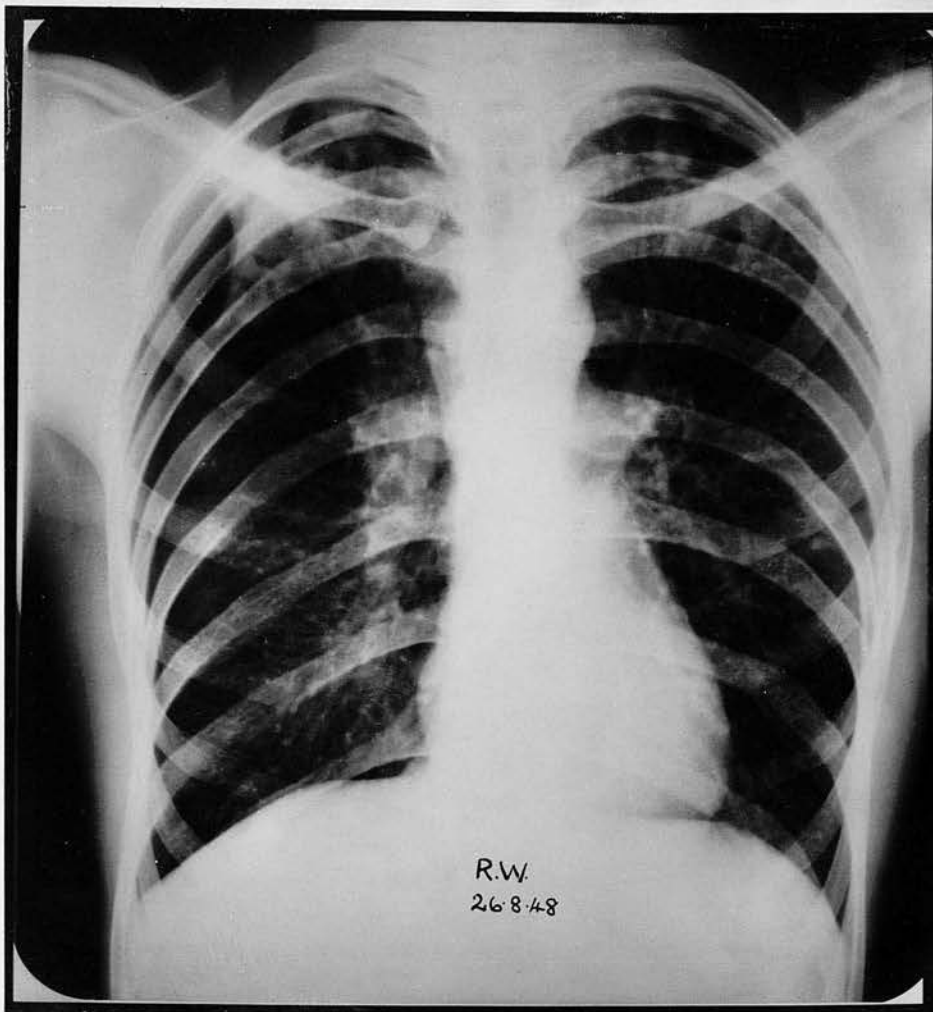


FIG. 34. R.W.  
Before treatment

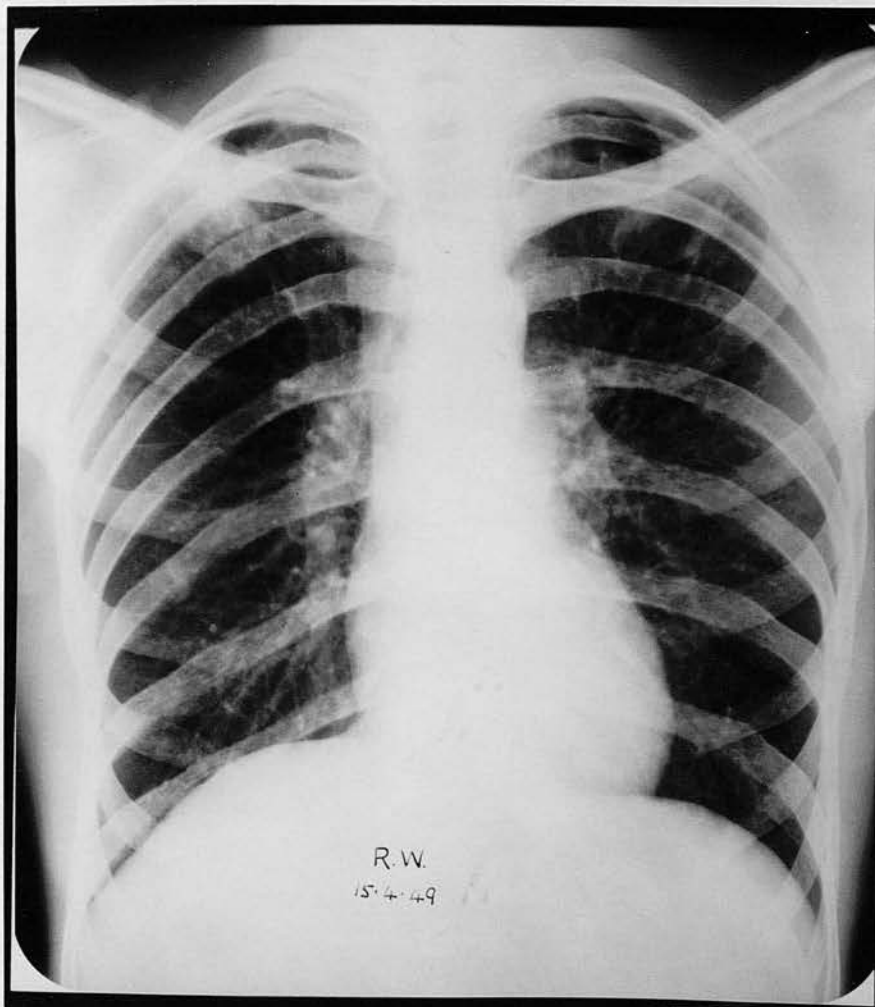


FIG. 35 R.W.  
After treatment.  
(22 weeks.)

Case 11. N.McK. A married woman of 27 who, three months before admission, had had a small haemoptysis. Bilateral pulmonary tuberculosis had been discovered and rest in bed prescribed.

When admitted she was pale and thin (weight 7st. 9lbs.) although no other signs of toxæmia were manifest. The X-ray appearances were as follows: Right lung: apical and infraclavicular mottling with signs of softening at anterior end of 1st rib. Left lung: apical upper and mid-zone mottling confluent in some areas but without definite excavation. Individual foci are small with ill-defined margins.

#### Treatment

A left artificial pneumothorax was induced and a reasonably satisfactory collapse was obtained despite indivisible apical adhesions. The right lung was kept under observation but when clinical evidence of activity appeared treatment with P.A.S. was instituted. (Fig. 36 ).

Length of course: 22 weeks

Dosage: 25 gm. daily

Signs of intolerance: nil

Average blood level: 5.8 mgm. per cent.

#### Progress under treatment

Considerable improvement in her general condition took place and 12 lbs. in weight was gained. The B.S.R. was already within normal limits when treatment/

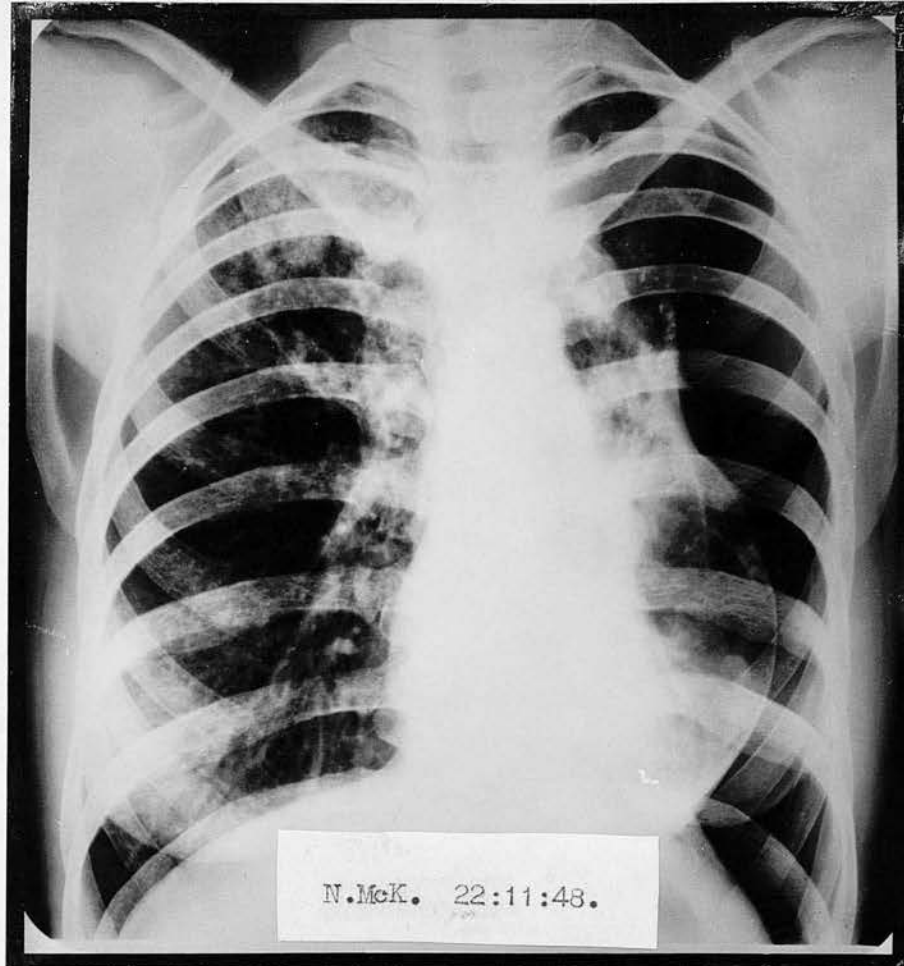


FIG. 36. N. McK.

Before P.A.S.

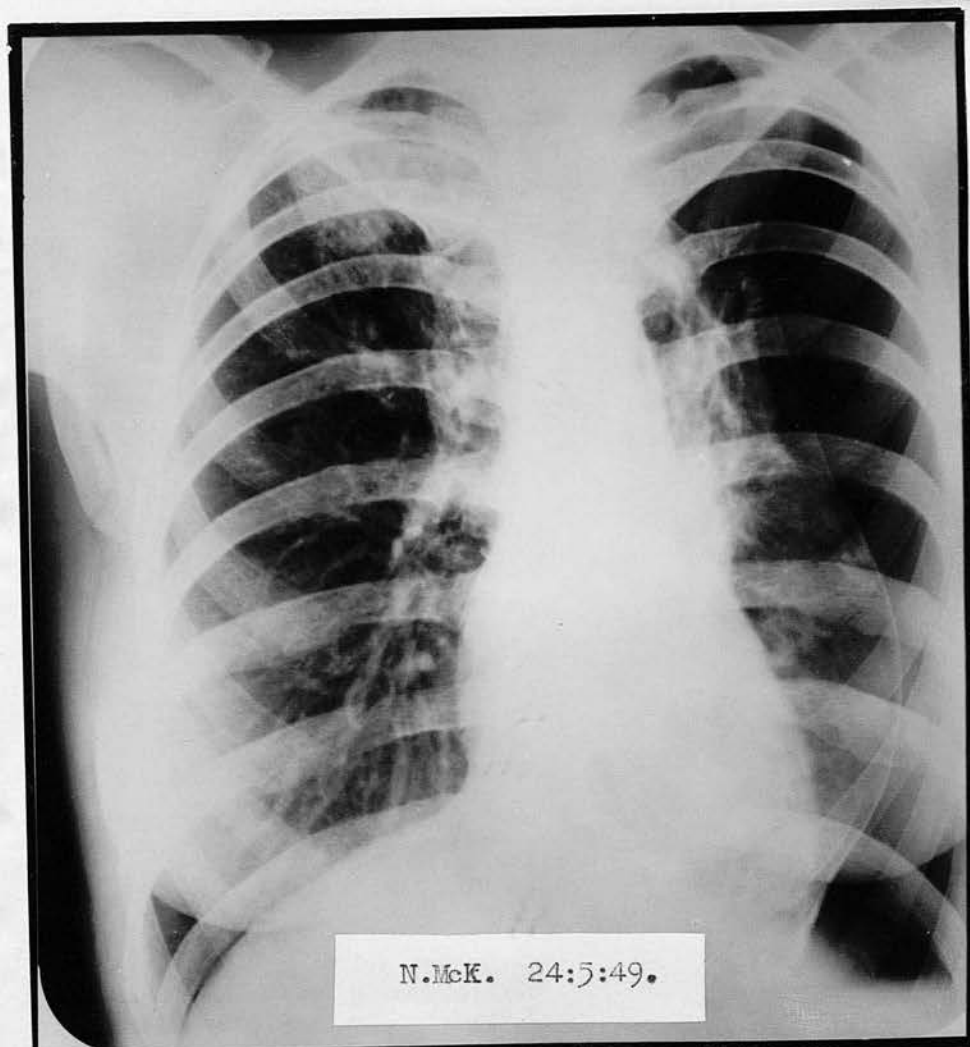


FIG. 37. N. McK.

After P.A.S.  
22 weeks.



Case 12. M.S. A young girl aged 17 who gave a history of bronchitis six months before admission. There was no other relevant feature in her history. A large thick-walled cavity was present occupying the main part of the upper lobe which had contracted pulling the lesser fissure upwards. Faint seeding was present below the cavity and in the left apex there was mottling with early softening. (Fig.38 )

Numberous tubercle bacilli were present in the sputum on direct smear.

#### Treatment

Treatment was limited to bed rest. Deterioration took place slowly with the formation of a second cavity in the right lung and the development of frank cavitation at the left apex. (Fig.39 ). At this stage, in the hope that chemotherapy would improve the condition of the left lung sufficiently to permit surgical intervention for the control of the disease in the right lung, a course of P.A.S. was given.

Length of course: 20 weeks

Dosage: 24 gm. daily

Signs of intolerance: Occasional sickness especially at the beginning of the course but this was not severe enough at any time to necessitate reduction of dosage.

Average blood level: 6.9 mgm. per cent.

#### Progress under treatment

No appreciable change took place in her general condition/

condition. A steady loss of weight was recorded amounting in all to 8 lbs. The B.S.R. readings fell from 30 to 22. Tubercle bacilli were still demonstrable on direct smear although in greatly reduced numbers.

Radiological changes: One month after commencement of treatment considerable reduction in the size of the right apical cavity was recorded (Fig. 40 ). The cavity before treatment measured 6 cm. x 4.5 cm., and after, 4 cm. x 2.5 cm. The lower cavity changed its shape because of the further contraction of the upper lobe, but was not reduced in size. That at the left apex was little altered. During the remaining months no further improvement was recorded, the appearances at the end of treatment being very similar to those in Fig. 40.

#### Summary

No change in general condition.

Reduction in size of cavity. Otherwise no striking change.

#### Conclusions

The reduction in the size of the cavity in this case was without doubt the result of P.A.S. therapy as the size had been unaltered by bed rest during the previous  $6\frac{1}{2}$  months. This did not however in any way alter or improve the serious therapeutic problem which this case presents.

#### Prognosis

Not materially changed by treatment.

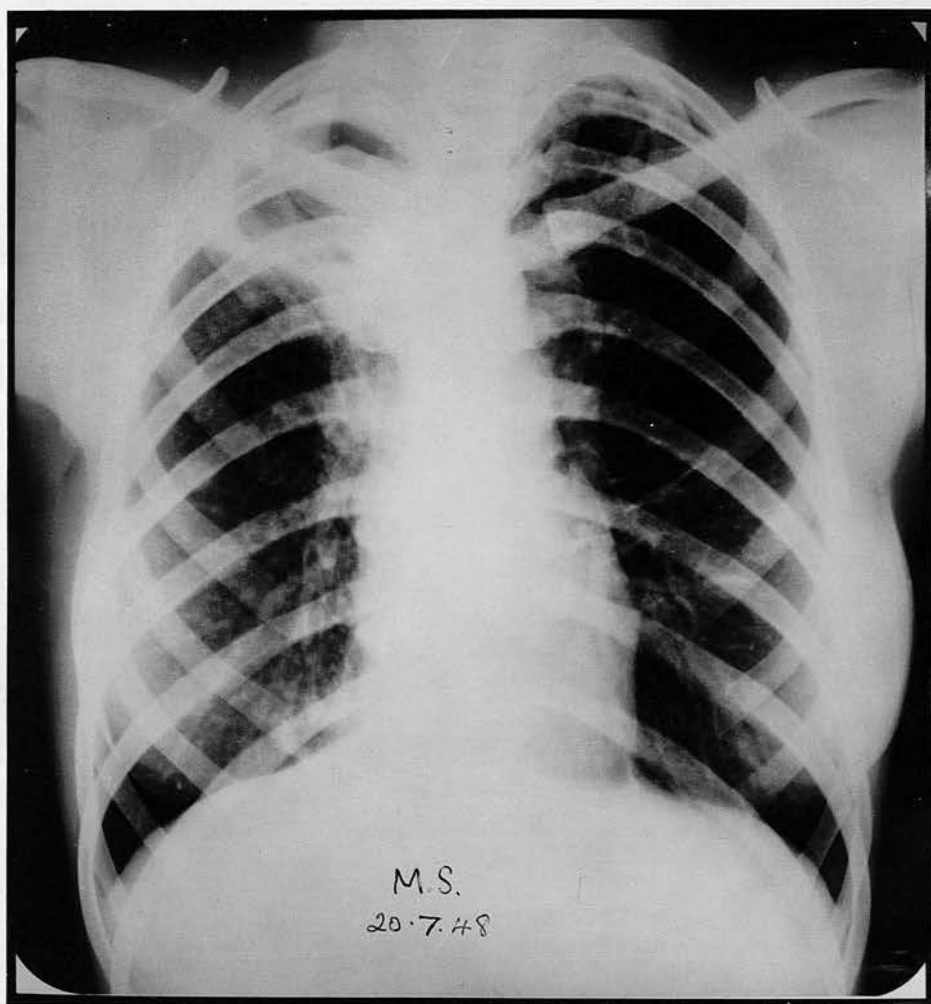


FIG. 38. M.S. On admission.

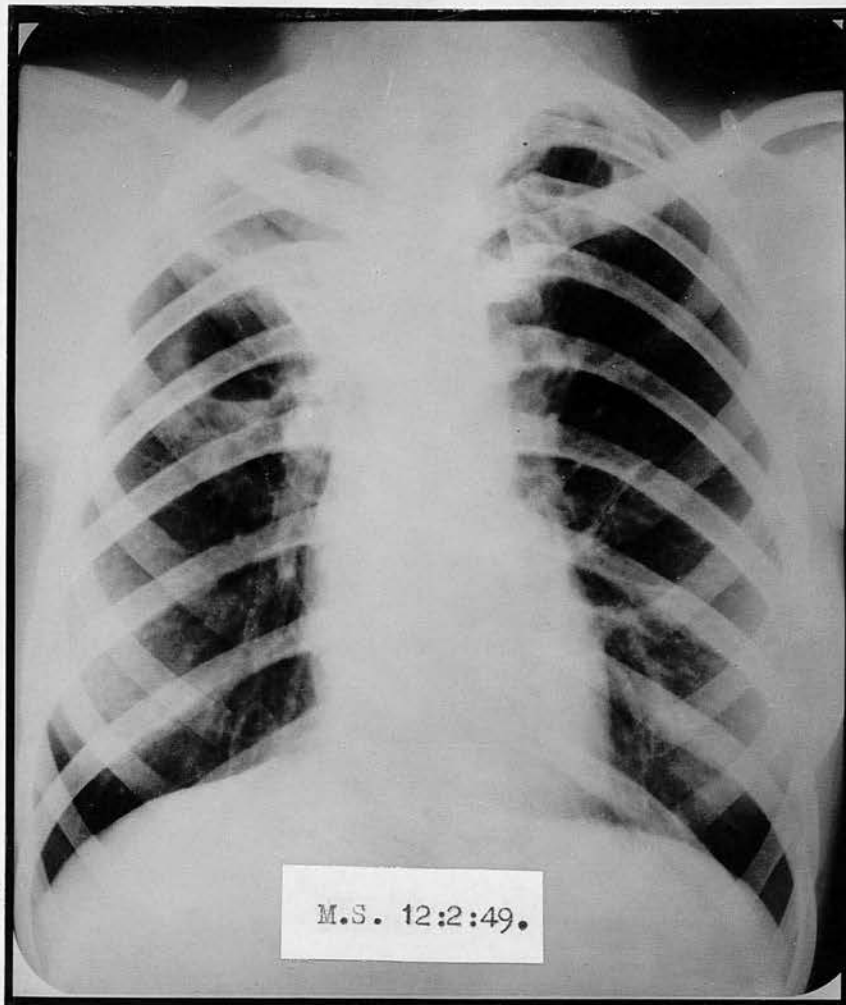


FIG.39. M.S.  
Before P.A.S.

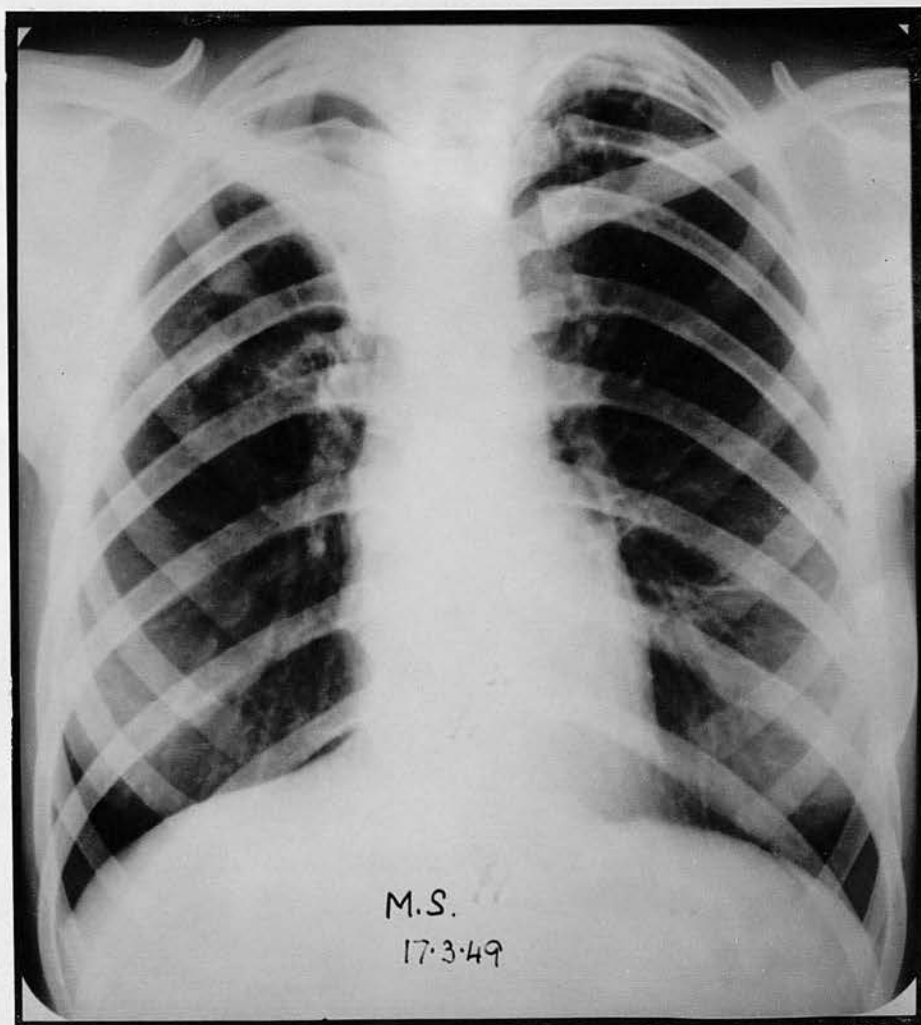


FIG.40. M.S.  
After 1 month  
treatment with  
P.A.S.



Case 13. M.O'B. A young woman of 21 who have a history of an attack of pleurisy one year before admission. Since then she had had a cough but it was not until a small haemoptysis occurred that attention was drawn to it. An interesting feature of the case is that the patient was also the subject of congenital heart disease which had been the source of a certain amount of dysphoea and slight cyanosis since early childhood.

When first seen widespread active tuberculosis was present, both lungs being involved. (Fig. 41 ). Two large cavities and one smaller cavity were present in the right lung with also two smaller ones in the left lung below the clavicle.

Tubercle bacilli were abundantly present in the sputum.

#### Treatment

A course of P.A.S. therapy was started immediately in the hope that the local condition might improve sufficiently to allow more active treatment later.

Length of course: 22 weeks

Dosage: 25 gm. daily

Signs of intolerance: occasional sickness only.

Average blood level: 5.9 mgm. per cent.

#### Progress under treatment

Initially a gain in weight with a fall in the B.S.R. readings were recorded but in the later months a gradual return to the original level took place/

place in both cases. There was over all a slight improvement in her general condition.

The number of tubercle bacilli in the sputum was not appreciably altered.

Radiological changes: At the end of three months treatment reduction in the size of all cavities had taken place. (Fig.42 ). In the right upper zone only one cavity was now clearly visible and this measured 3 cm. x 2.5 cm. compared with the original cavities in this region which measured 4.5 cm. x 4 cm. and 2.5 cm. x 2.5 cm. respectively. A certain amount of resolution had also occurred in the infiltrative lesions. At the end of treatment (Fig. 43) a little further resolution had occurred but the cavities had again increased in size, although with the exception of that at the periphery of the right lower zones which was larger than the original, not to their size initially. Thus the greatest improvement was noted in the first three months of treatment.

#### Summary

Slight improvement in general condition.

Reduction in size of cavities with some resolution radiologically.

#### Conclusions

The benefit which accrued from the P.A.S. therapy was not of sufficient degree, despite good tolerance, to allow of, at any time, further treatment. This type of advanced disease presents an impossible/

impossible task for any form of therapy and it is not surprising that P.A.S. did not produce lasting benefit.

Prognosis

Unchanged by treatment.

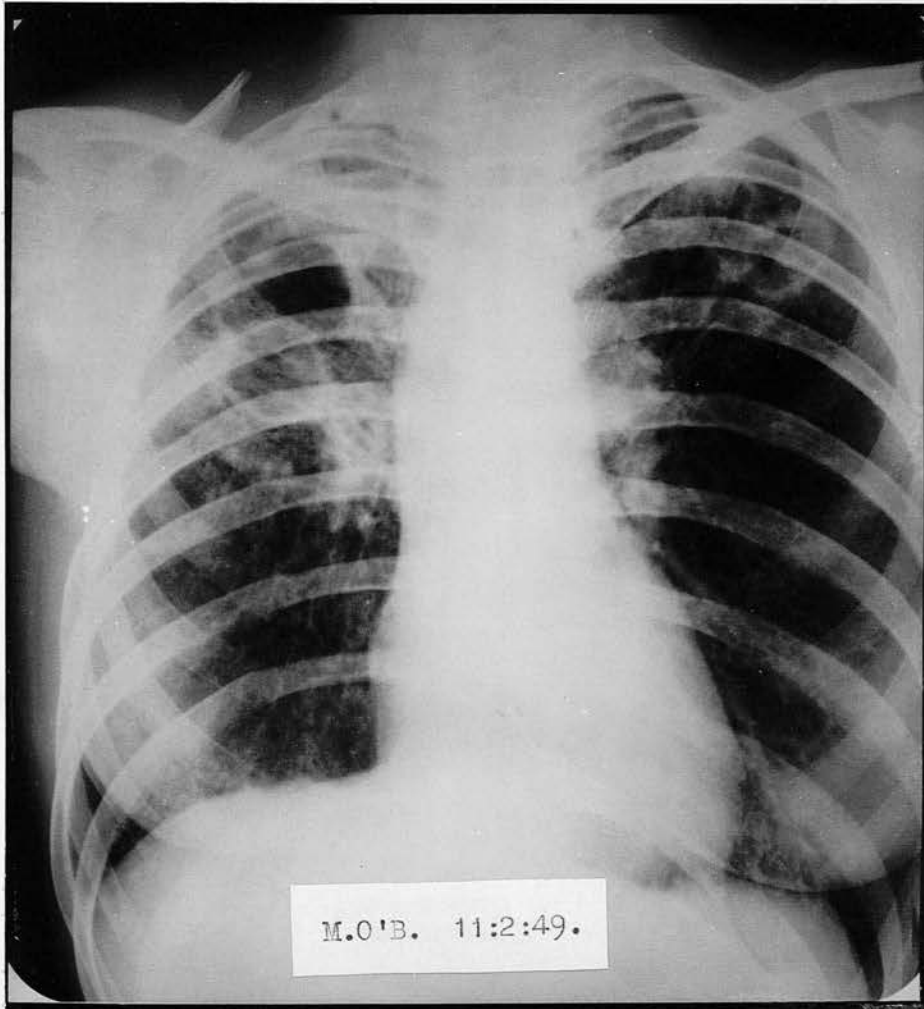


FIG. 41. M.O'B. On admission.

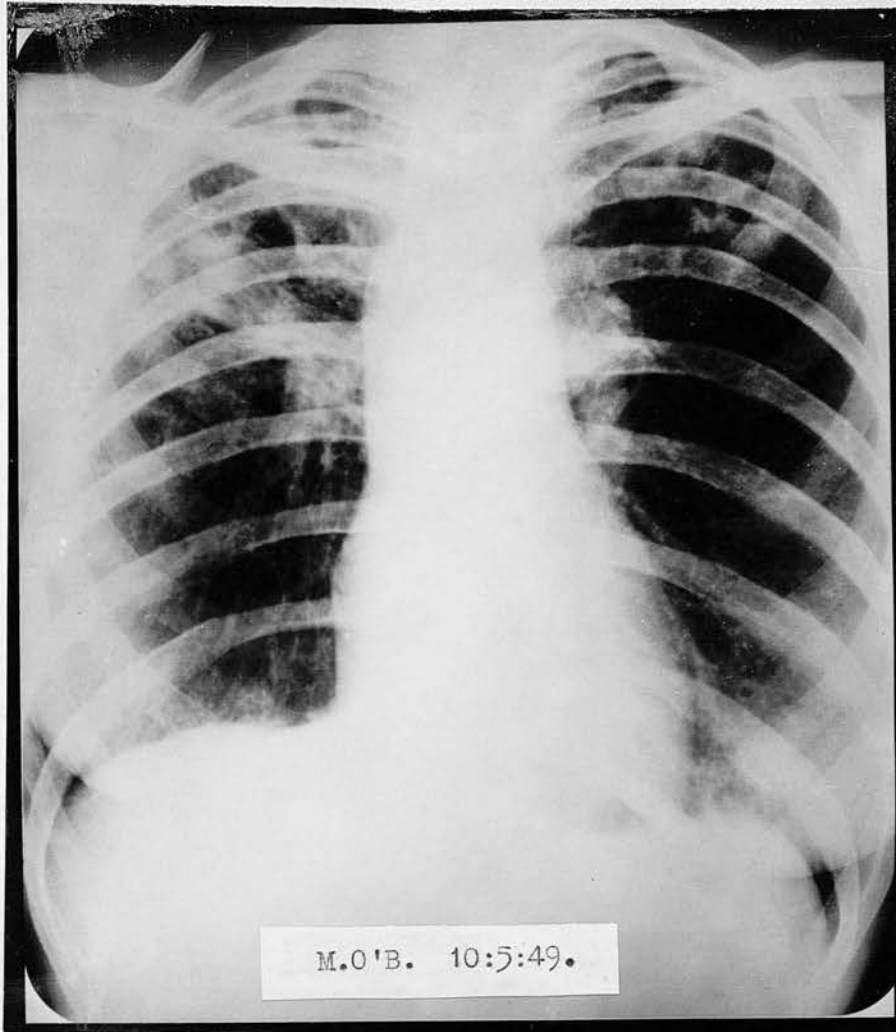


FIG. 42. M.O'B.  
After 3 months  
P.A.S.

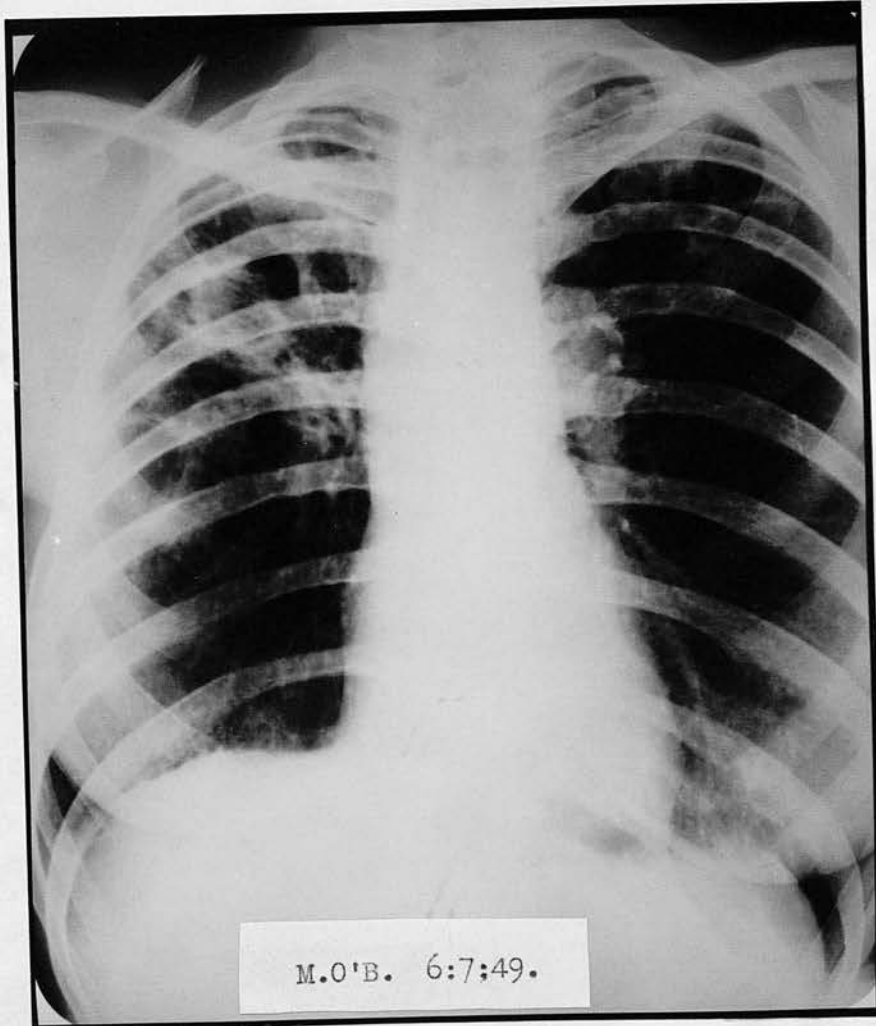


FIG. 43. M.O'B.  
At end of  
treatment.  
(22 weeks.)



Case 14. W.F. A man aged 29 who had been complaining of a cough for nine months before he was first seen. Extensive, bilateral and active pulmonary tuberculosis was discovered (Fig. 44 ). A cavity 4.4 cm. x 3 cm. was present in the right lung and excavation was commencing below the left clavicle. Tubercle bacilli were present in moderate numbers in the sputum.

#### Treatment

A course of P.A.S. was instituted immediately on admission as the disease was too extensive for collapse therapy.

Length of course: 23 weeks

Dosage: 25 gm. daily

Signs of intolerance: nil

Average blood level: 3.1 mgm. per cent.

#### Progress under treatment

Appreciable improvement took place in his general condition.  $7\frac{1}{2}$  lbs. in weight were gained, the major part during the first three months. The B.S.R. readings fell from 110 to 31 although considerable fluctuation occurred.

Tubercle bacilli continued to be demonstrable on direct smear in the sputum.

Radiological changes: These consisted in reduction in the size of both cavities. After three months treatment (Fig. 45 ) the right infraclavicular cavity had diminished from 4.4 cm. x 3 cm. to 2.8 cm. x 1.5 cm. Resolution and hardening of the individual/

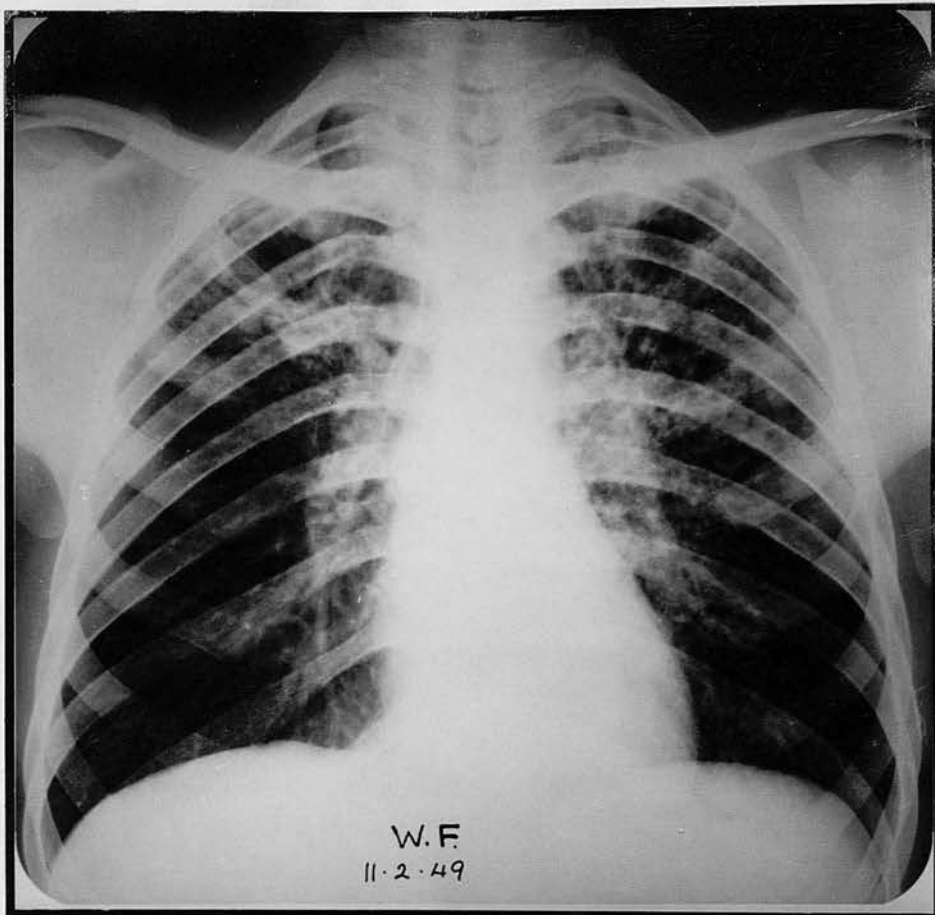


FIG.44. W.F.  
On admission.

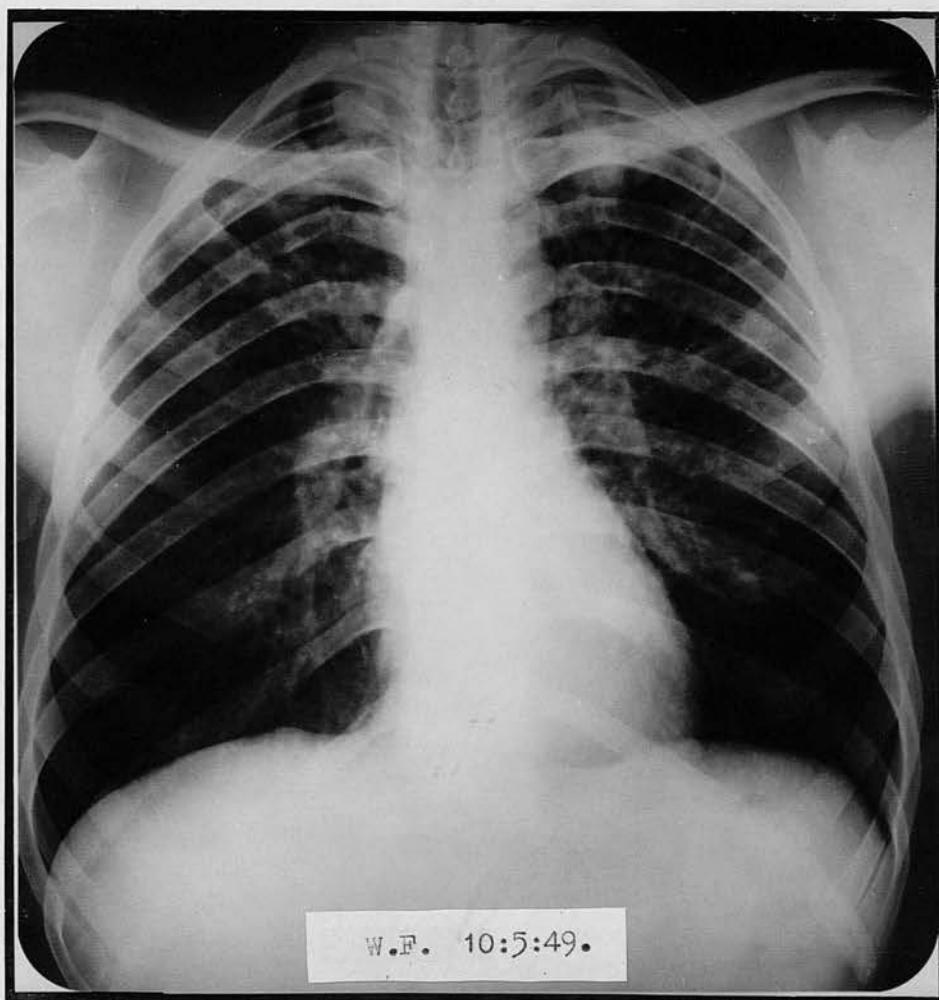


FIG.45. W.F.  
After 3 months  
P.A.S.

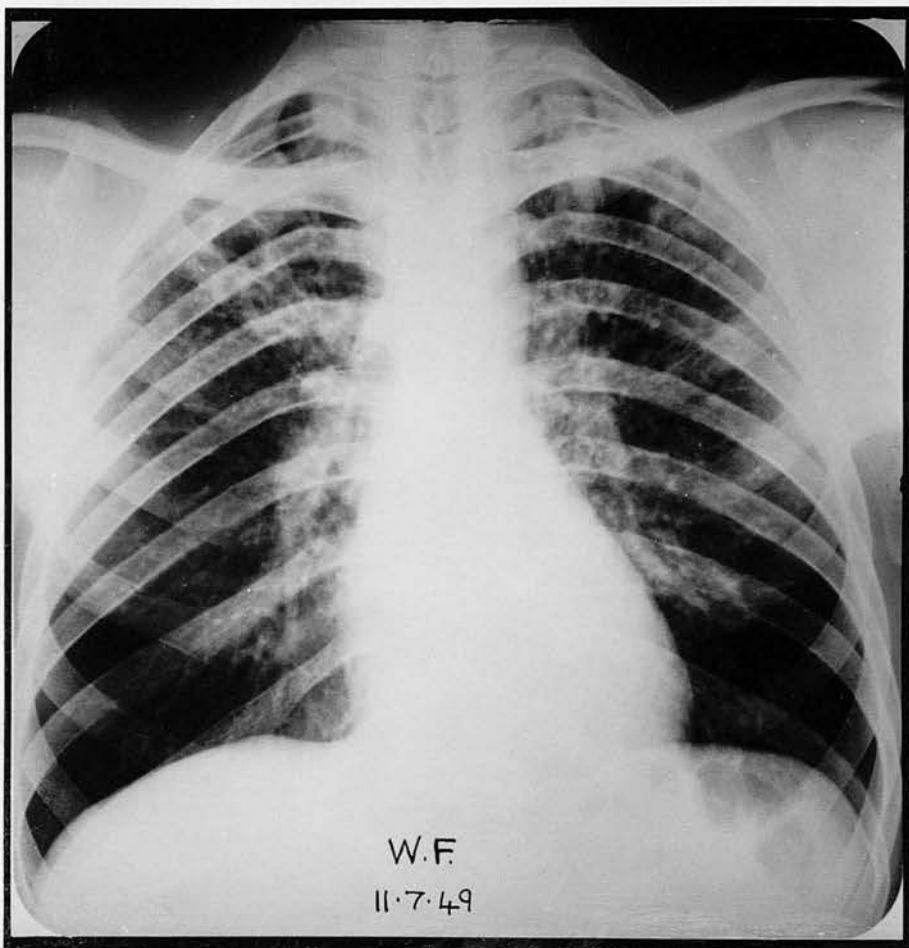


FIG. 46. W.F. After 22 weeks treatment.

Case 15. J.K. A man of 30 in whom the presenting symptom was haemoptysis occurring two months before admission. A similar happening eighteen months previously had been disregarded.

On admission active tuberculosis was present in both lungs involving the apex, upper and mid-zones of the left lung and the apex and upper zones of the right lung. (Fig. 47). No definite excavation was present but there was a suggestion of early softening below the left clavicle.

Tubercle bacilli were present in considerable numbers in the sputum.

#### Treatment

Bilateral pneumothorax was at first contemplated but, when a deselective collapse was obtained on the left side, this course was perforce abandoned. It was then decided to supplement bed rest with a course of P.A.S.

Length of course: 22 weeks

Dosage: 25 gm. daily

Signs of intolerance: nil

Average blood level: 4.7 mgm. per cent.

#### Progress under treatment

Marked improvement occurred in his general condition. 1 stone 2 lbs. in weight were gained. The B.S.R. readings fell from 10 to 2 within one month and have remained at that level.

Culture results from the sputum became negative for tubercle bacilli.

Radiological/





FIG. 47. J.K.

Before P.A.S.

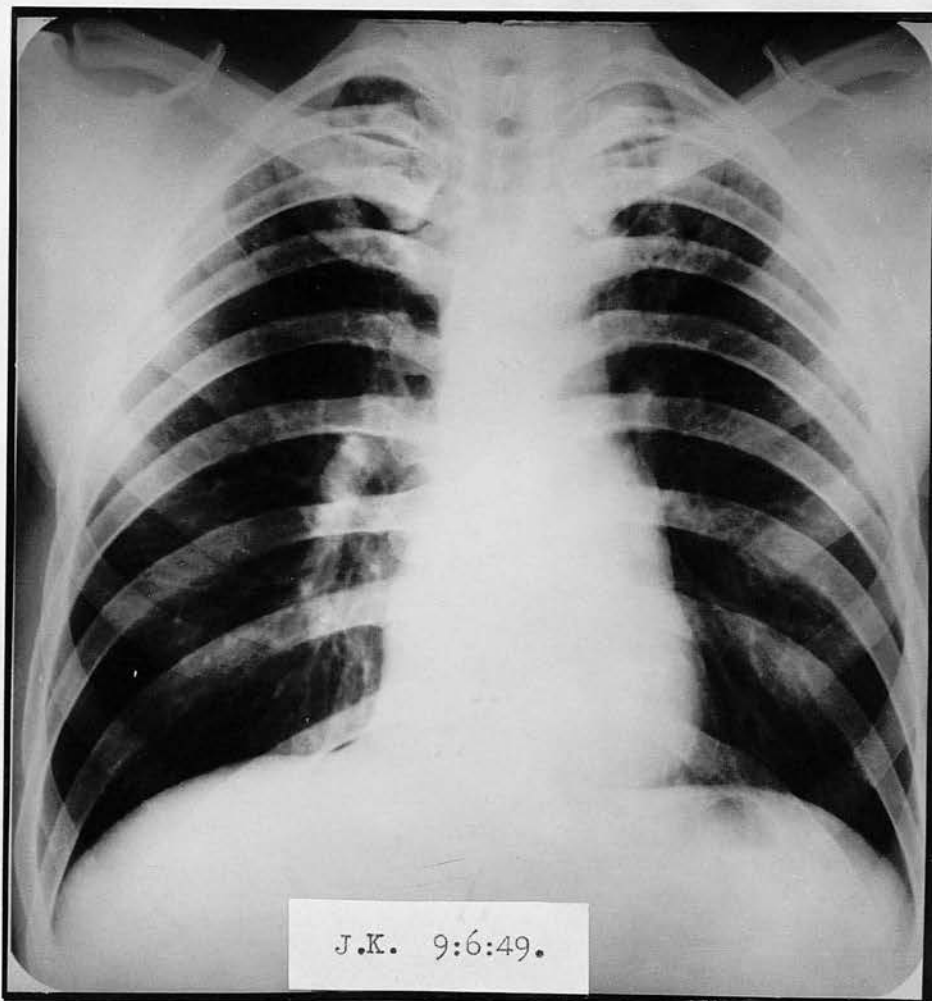


FIG. 48. J.K.

After 4 months  
P.A.S.

Case 16. U.H. A young woman aged 20 who gave a history of six months productive cough and periodic huskiness of the voice. X-ray examination revealed widespread active tuberculosis. The apex and upper zone of the right lung was largely excavated and in the mid-zone mottling with several smaller cavities was present. The disease in the left lung consisted in apical, upper and mid-zone mottling without excavation. The patient was thin and toxic looking with a malar flush. Tubercle bacilli were abundantly present in the sputum.

#### Treatment

After a month's bed rest, from which no benefit accrued it was decided to try the effect of a course of P.A.S.

Length of course: 20 weeks

Dosage: 24 gm. daily

Signs of intolerance: Occasional sickness especially at beginning of course.

Average blood level: 5.6 mgm. per cent.

#### Progress under treatment

During a short initial period slight improvement in the general condition occurred with fall in B.S.R. and gain in weight. Steady deterioration then began. 8 lbs. weight was lost in all and the B.S.R., after fluctuating, returned to its previous level. Numerous tubercle bacilli were still present in the sputum.

Radiological appearances: Figs. 49 & 50 illustrate/

trate the radiological appearances before and after treatment. All cavities have increased slightly in size and the infiltrative disease in the left lung has become rather more extensive.

#### Summary

Deterioration in local and general condition.

#### Conclusions

P.A.S. therapy has been ineffective in checking the expected course of the disease in this patient.

#### Prognosis

Unchanged by treatment.

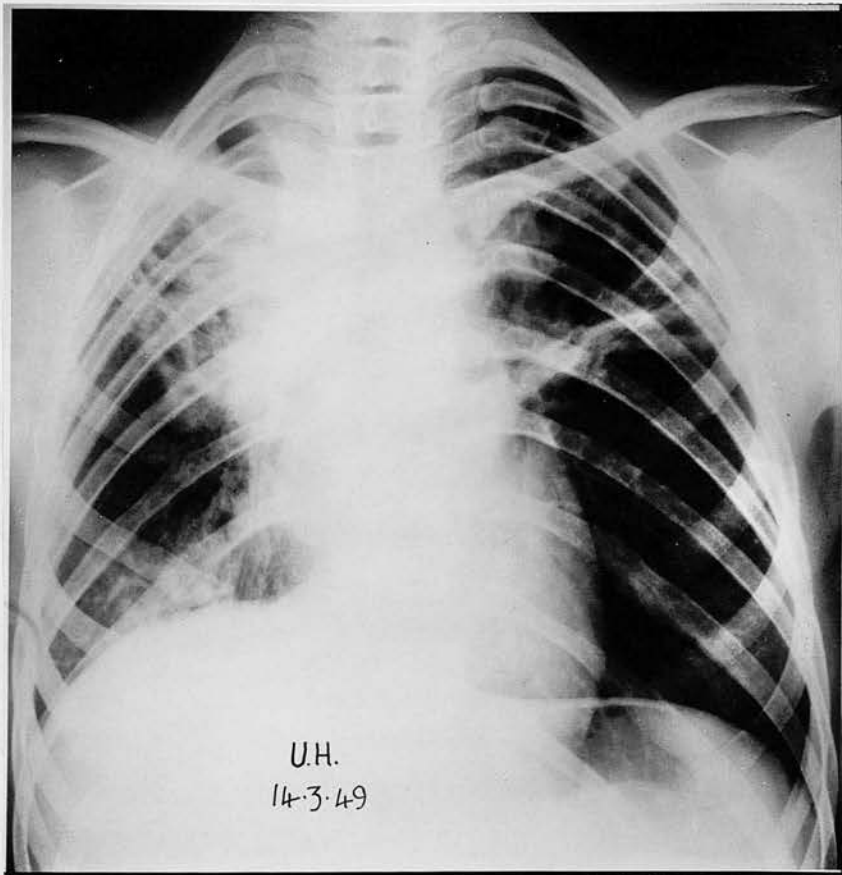


FIG. 49. U.H.

Before P.A.S.

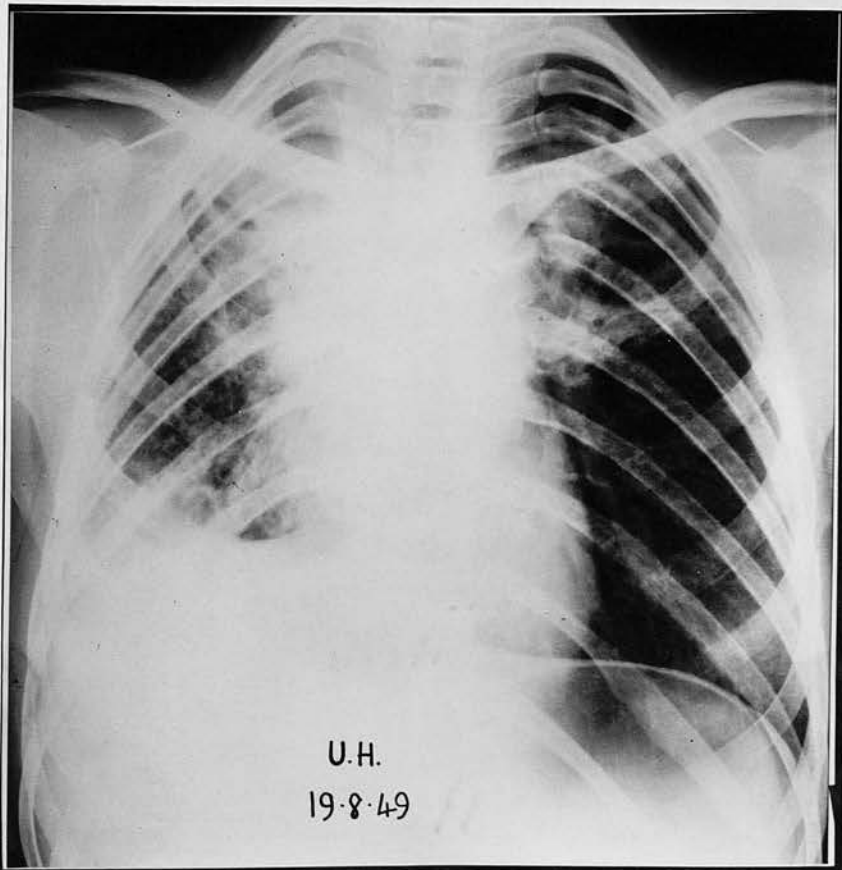


FIG. 50. U.H.

After P.A.S.

(20 weeks.)



Case 17. M.McA. A young girl of 17 who gave a history of cough of two months' duration with pain in the chest and breathlessness for three weeks before admission. One feature in her past history is worthy of note, namely that at the age of six she had undergone radical dissection of the glands of the neck.

When first seen she was pale, toxic looking and thin and the X-ray film revealed bilateral pulmonary tuberculosis, the disease being recent and active. The lesions consisted in mottling in the left apex, upper and mid zones, densest in the mid zone where cavity formation had taken place. Mottling was also present at the right apex and in the upper zone. Tubercle bacilli were present in large numbers in the sputum on direct smear.

#### Treatment

Following a short period of bed rest, during which temperature disturbance present on admission began to settle, it was decided that this was the type of case in which a course of P.A.S. would in all likelihood prove beneficial. Fig.51 illustrates the X-ray appearances before the start of treatment.

Length of course: At the time of writing the patient had been under treatment with P.A.S. for 13 weeks. It is proposed to continue with the drug for a further four weeks.

Dosage: 25 gm. daily

Signs/

Signs of intolerance: nil

Average blood level: 3.8 mgm. per cent.

#### Progress under treatment

Steady improvement occurred in all findings and was particularly marked in the general condition. The temperature range fell to within normal limits, physical signs of activity steadily diminished until they had completely disappeared  $3\frac{1}{2}$  months after the start of treatment. The B.S.R. reading fell from 70 to 7 and a gain in weight of 1 stone took place within four months. After three months' treatment no growth was obtained on culture of the sputum.

Radiological appearances: Resolution of the lesions in both lungs was demonstrated to be occurring steadily from month to month and Figs. 52 and 53 illustrate the appearances one month and three months after the start of treatment. Tomography of the left lung confirmed the closure of the original cavity in this lung.

#### Summary

Marked improvement in general condition.

Considerable resolution in lung lesions.

#### Conclusions

It is considered that the rapidity and extent of the clearing of the lesions in this patient must be due in large part to the action of P.A.S.

#### Prognosis

Improved under treatment.

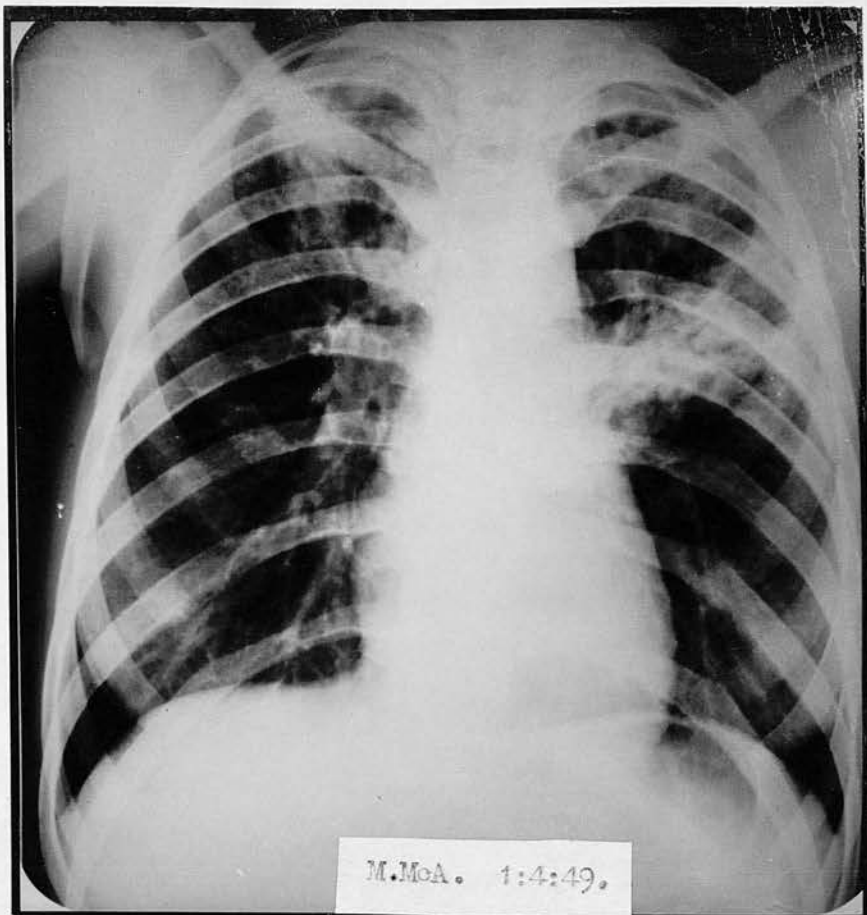


FIG. 51. M. McA.

Before P.A.S.

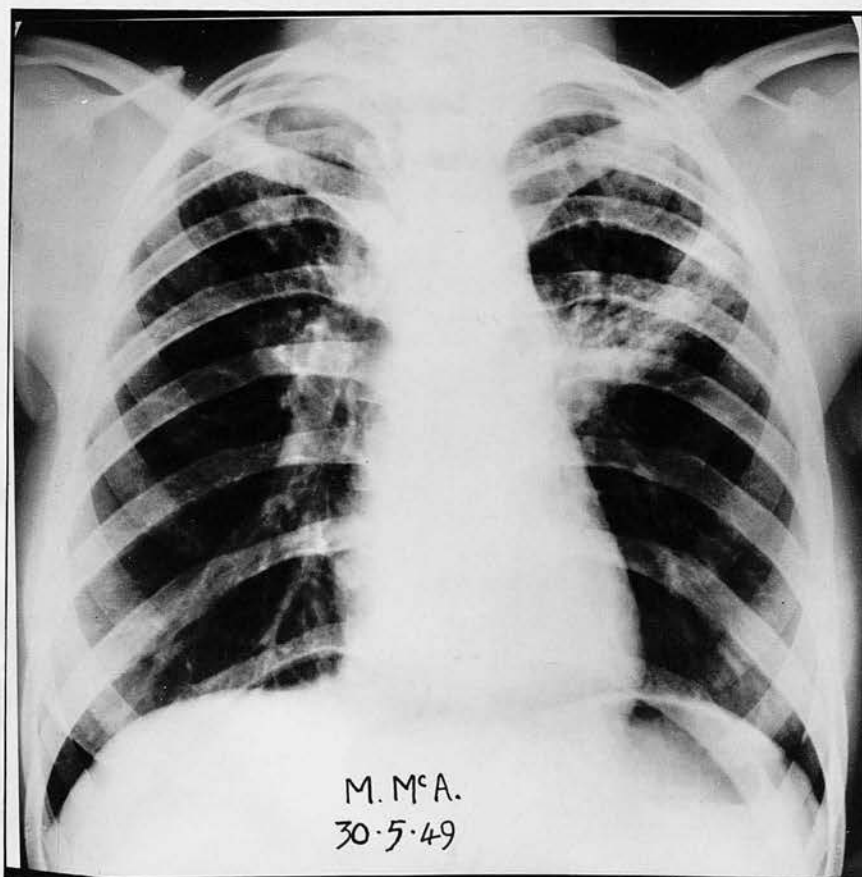


FIG. 52. M. McA.

After 1 mth's

P.A.S.

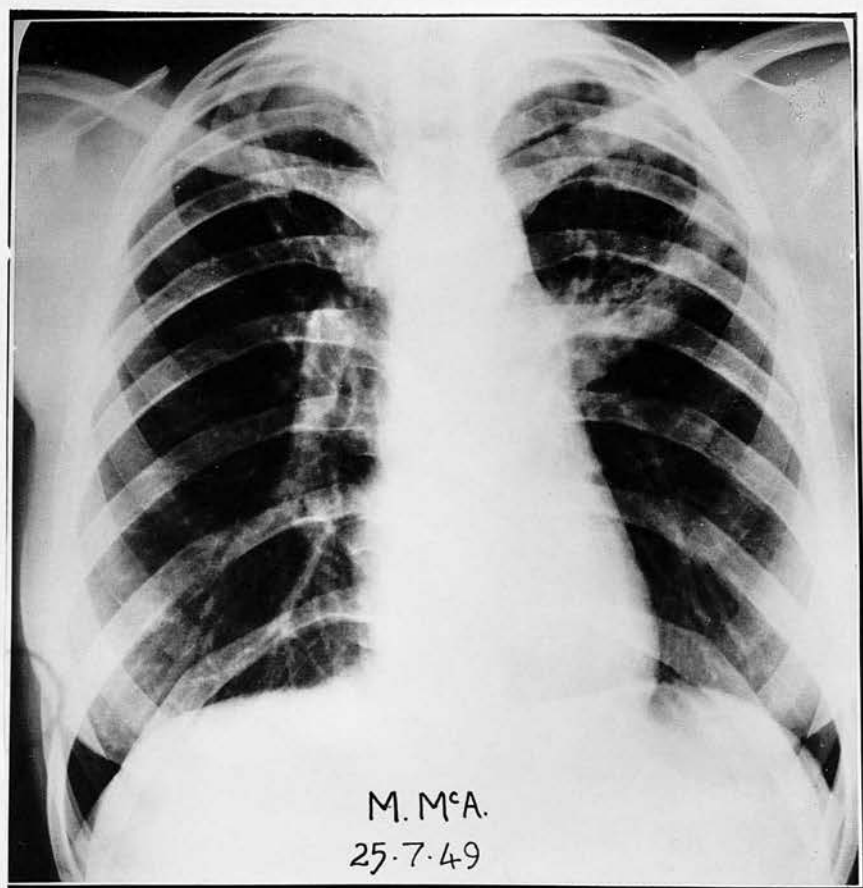


FIG. 53. M.McA. After 3 month's P.A.S.



Case 18. M.C. A young girl of 17 who had been under treatment for over two years when the decision to start a course of P.A.S. therapy was made.

When first admitted bilateral pulmonary tuberculosis was present. In addition to active, excavating disease in the lungs, the patient was the subject of early active tuberculosis of the left ankle joint. In the course of two years' immobilisation apparent radiological healing of the joint by ankylosis took place. During this time, following an unsuccessful attempt at artificial pneumothorax, gradual deterioration had taken place in the pulmonary lesions, notably an increase in excavation in the right lung. Nevertheless the disease had not become as extensive as might have been expected in this time and, in view of the healing powers evidenced in the ankle joint, a course of P.A.S. was decided upon. It was hoped by this means that sufficient improvement would occur to permit further surgical measures later.

Fig. 54 illustrates the radiological appearances before the commencement of treatment.

Tubercle bacilli were demonstrated on gastric lavage, as no sputum was being produced at this time.

#### Treatment

P.A.S. therapy.

Length of course: At the time of writing the patient had been under treatment with P.A.S. for

13 weeks. The course is being continued.

Dosage: 24 gm. daily

Signs of intolerance: nil

Average blood level: 6 mgm. per cent.

#### Progress under treatment

Some improvement in general condition with a gain of weight of 4 lbs. The B.S.R. readings fell from 114 to 49. An interesting feature of this case is that before treatment with P.A.S. began albumen was present in the urine to the extent of 4 - 5 parts (Esbach). After four months treatment this had fallen to  $\frac{1}{2}$  part (Esbach).

Tubercle bacilli were still demonstrable on gastric lavage.

Radiological changes: Fig. 55 illustrates the X-ray appearances three months after treatment started. Although some resolution of the infiltrative lesions has occurred, the most striking change is the diminution in the cavitation in the right lung. This reduction in size was evident fourteen days after the commencement of treatment and is being maintained. The present position is as illustrated. The size of the original cavities were 5 cm. x 5.2 cm. and 3 cm. x 3.4 cm. respectively. The present measurements are 3.5 cm. x 4.3 cm. and 2.5 cm. x 3 cm.

#### Summary

Slight/

Slight improvement in general condition.

Radiologically, reduction in extent of excavation.

#### Conclusions

Radiological evidence is available to show that before treatment excavation in this patient was gradually increasing. There can be little doubt, therefore, that P.A.S. was operative in reducing the size of the cavities. They are still, however, of considerable size and it would seem that little further reduction can be expected. The therapeutic problem has not, therefore, been materially affected by the treatment despite the improvement recorded.

#### Prognosis

Little changed by treatment.

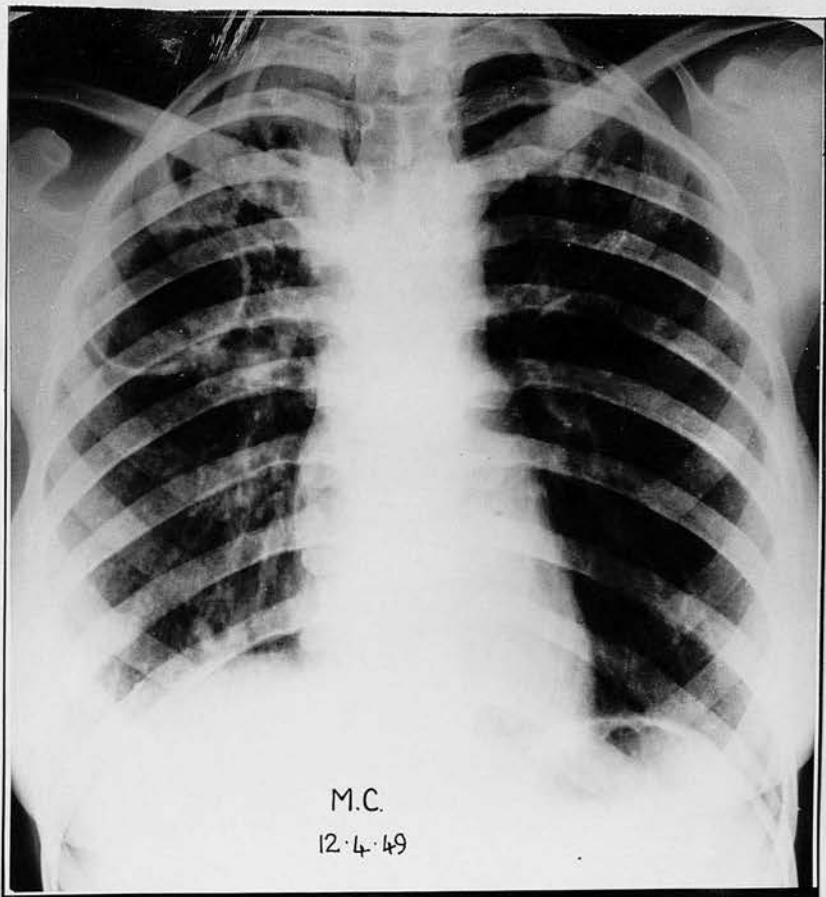


FIG. 54. M.C.

Before P.A.S.

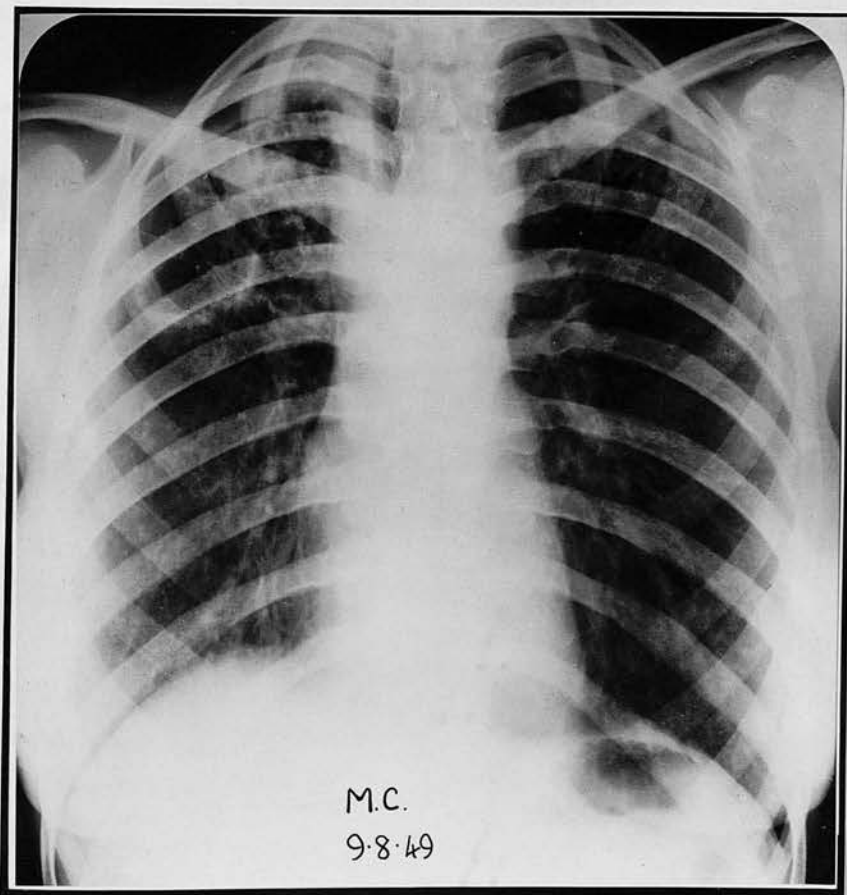


FIG. 55. M.C.

After 3 mth.'s  
P.A.S.



Case 19. J.McC.A young woman of 20 in whom a cough with loss of weight of three months' duration culminated in an illness at first regarded as influenza but later proved to be active tuberculosis. A highly active infiltration with commencing cavitation was found to be present in the right upper zone. There was also a recent spread to the mid zone of the contralateral lung.

Tubercle bacilli were demonstrated in the sputum on direct smear.

#### Treatment

A right artificial pneumothorax was induced but this resulted in an atelectatic lobe and a tension cavity, necessitating abandonment of this type of treatment.

Treatment with P.A.S. was instituted at this stage and Fig. 56 illustrates the pulmonary condition at this time.

Length of course: At the time of writing 17 weeks treatment had been given. The course is being continued.

Dosage: 24 gm. daily

Signs of intolerance: nil

Average blood level: 6.6 mgm. per cent.

From the 4th to the 10th week of the P.A.S. course the drug was supplemented with streptomycin 1 gm. daily (50 days). In the 14th week, too, a pneumoperitoneum was induced.

#### Progress/

### Progress under treatment

Marked improvement occurred in her general condition, gross temperature disturbance (range 99° - 103°F) subsiding to within normal limits in the course of the first four weeks. Gain in weight of 2 lbs. and a fall in the B.S.R. readings from 66 to 4 were recorded.

The sputum became negative for tubercle bacilli on direct smear three months after the start of treatment. The result of culture is not yet to hand.

Radiological changes: Figs. 57 and 58 illustrate the radiological appearances 1½ months and 3½ months after treatment started. The right apical cavity became gradually smaller and in Fig. 58 appears to have closed. A series of tomograms taken at this time revealed only a very small irregular area of residual cavitation. Decrease in density in the opacity was also noted. The small infiltration in the left lung showed partial resolution and contraction.

### Summary

Improvement in general condition.

Improvement in radiological findings.

### Conclusions

This patient was seriously ill when admitted and the resultant improvement is greater than could have been expected without special measures. It is felt that despite the combination of streptomycin with/

with the P.A.S. there is clear evidence of the beneficial effect of the latter drug.

Prognosis

Improved as a result of treatment.

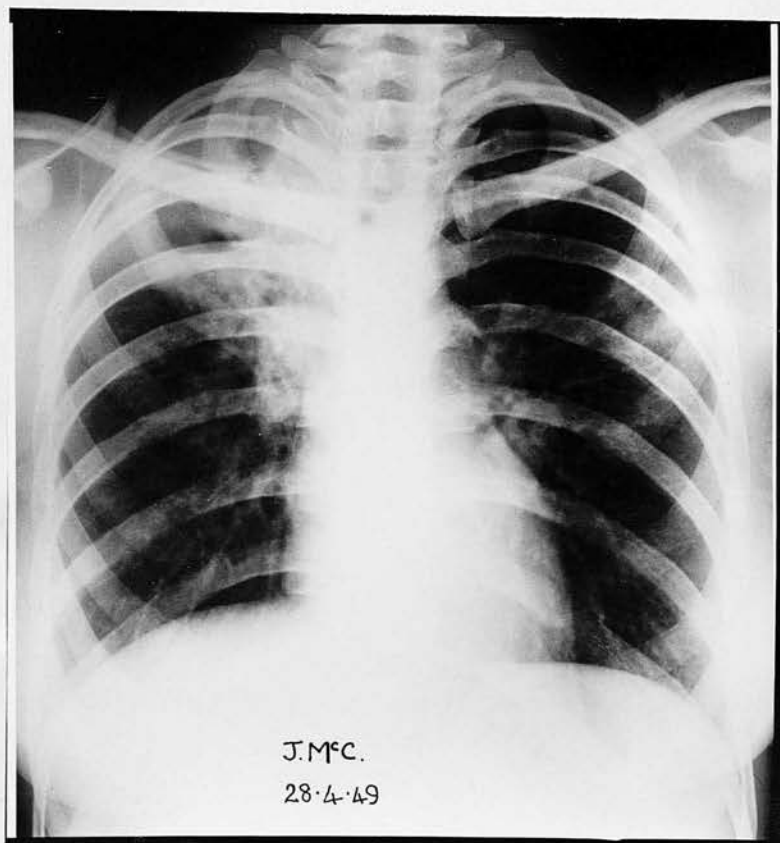


FIG. 56.     J.McC.     Before P.A.S.

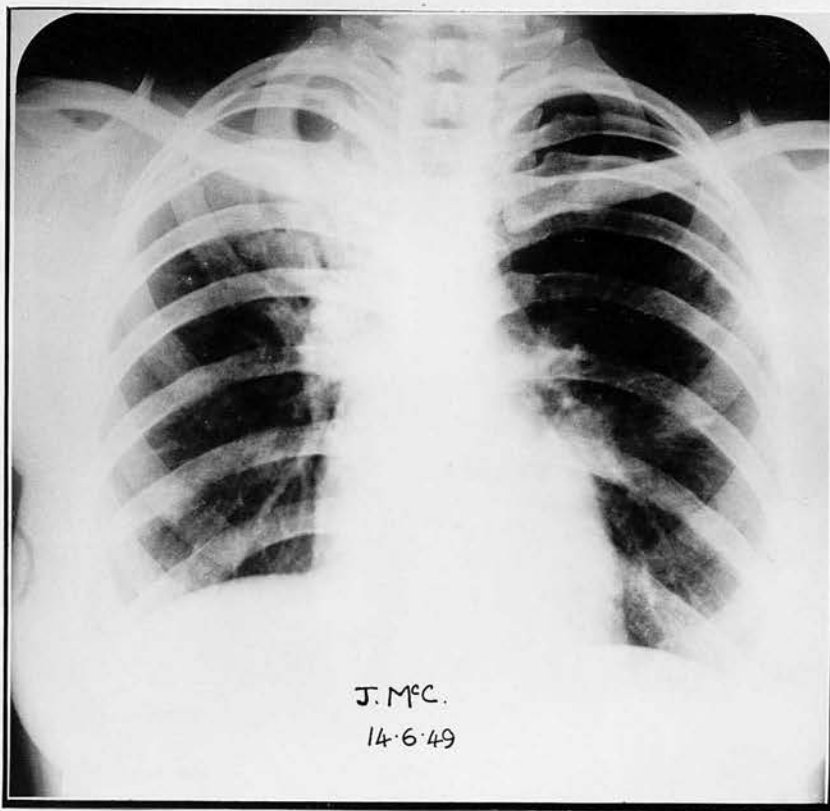


FIG. 57. J. McC  
After  $1\frac{1}{2}$  mths.  
P.A.S.

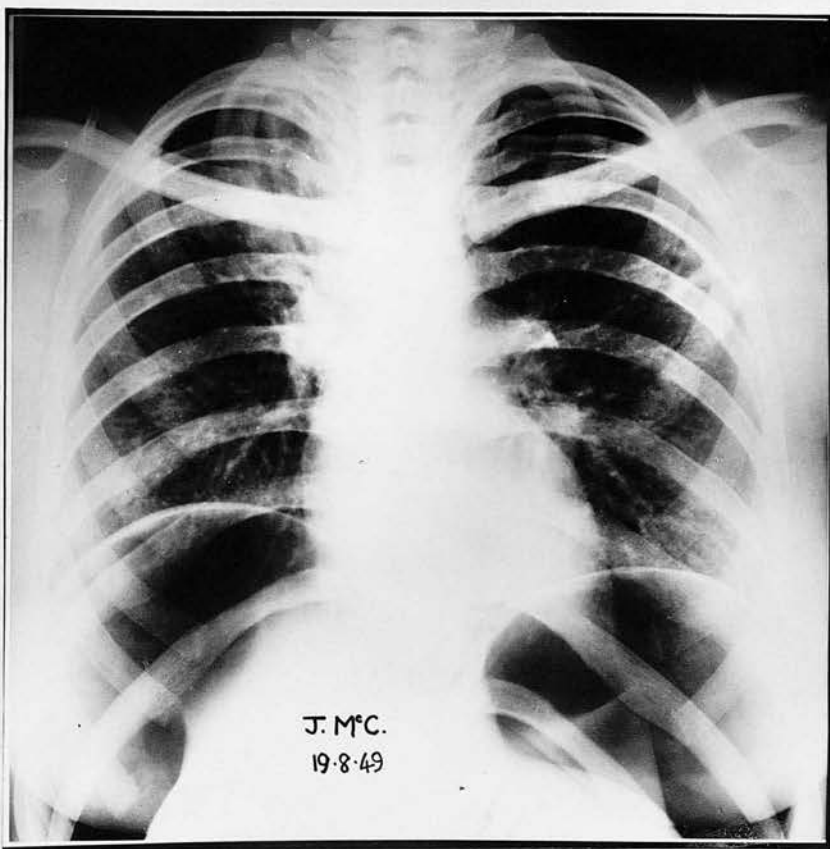


FIG. 58. J. McC.  
After  $3\frac{1}{2}$  mths.  
P.A.S.



Case 20. J.W. A man aged 35 in whom the presenting symptom was again haemoptysis, although on inquiry a six months' history of cough was elicited.

The patient was pale and thin, weight 3 st. 5 lbs.

Tubercle bacilli were present in the sputum in large numbers and the X-ray picture revealed extensive fibro-caseous disease in the right lung with a recent infiltration at the base of the contralateral lung.

#### Treatment

After two months' treatment on bed rest alone it was decided to give this patient a course of P.A.S. as deterioration was occurring in the left basal lesion and it was hoped by this means to improve his condition sufficiently to permit surgical collapse of the right lung. Fig.59 illustrates the state of the lesion at this time.

Length of course: At the time of writing a course of 15 weeks had been given and the course was continuing.

Dosage: 20 gm. daily. This was found to be the upper limit of the patient's tolerance of the drug.

Signs of intolerance: Vomiting and diarrhoea especially troublesome towards the end of each weekly course.

Average blood level: 5.5 mgm. per cent.

#### Progress/

Progress under treatment

No change in general condition. B.S.R. readings, after an initial fall, have returned almost to the original figure (66 to 55). 4 lbs. in weight has been lost and tubercle bacilli are still abundantly present in the sputum.

Radiological changes: Fig. 60 illustrates the X-ray appearances after three months' treatment. Some resolution of the infiltrative lesions has occurred in both lungs and the individual foci are harder. Some change in the shape of the numerous cavities in the right lung has occurred but there is no appreciable reduction in size.

Summary

No improvement in general condition.

Slight improvement in radiological appearances.

Conclusions

The result here has been disappointing and it is not anticipated that much further improvement will occur as a result of persistence with the treatment.

Prognosis

Unaltered by treatment.

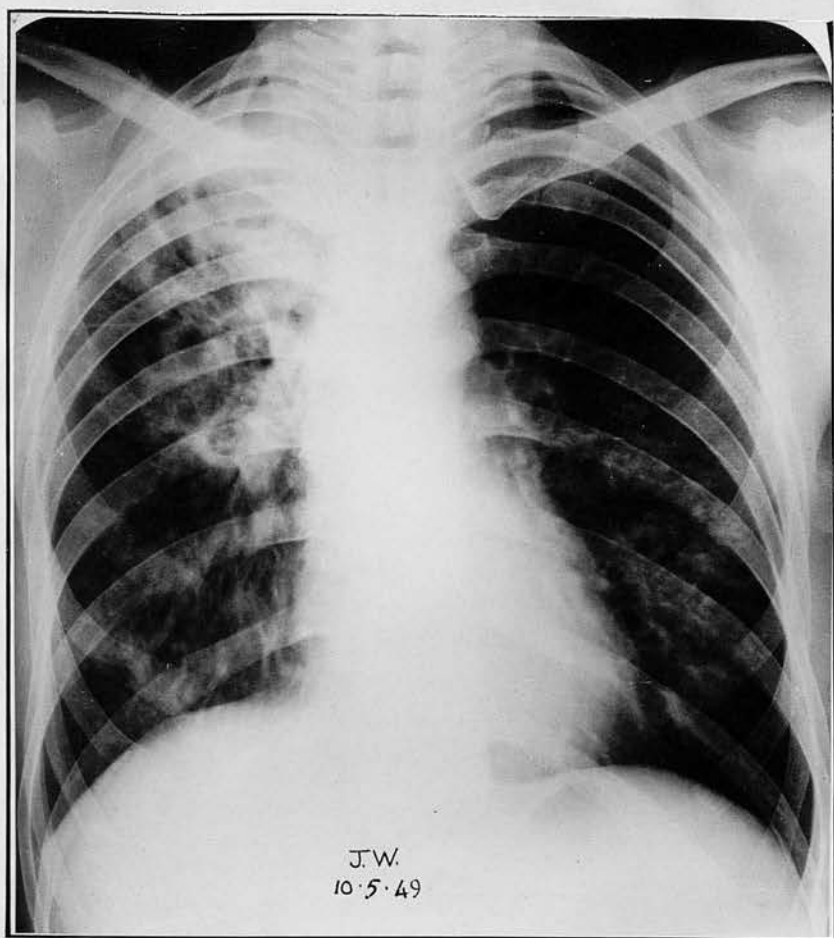


FIG. 59. J.W.

Before P.A.S.

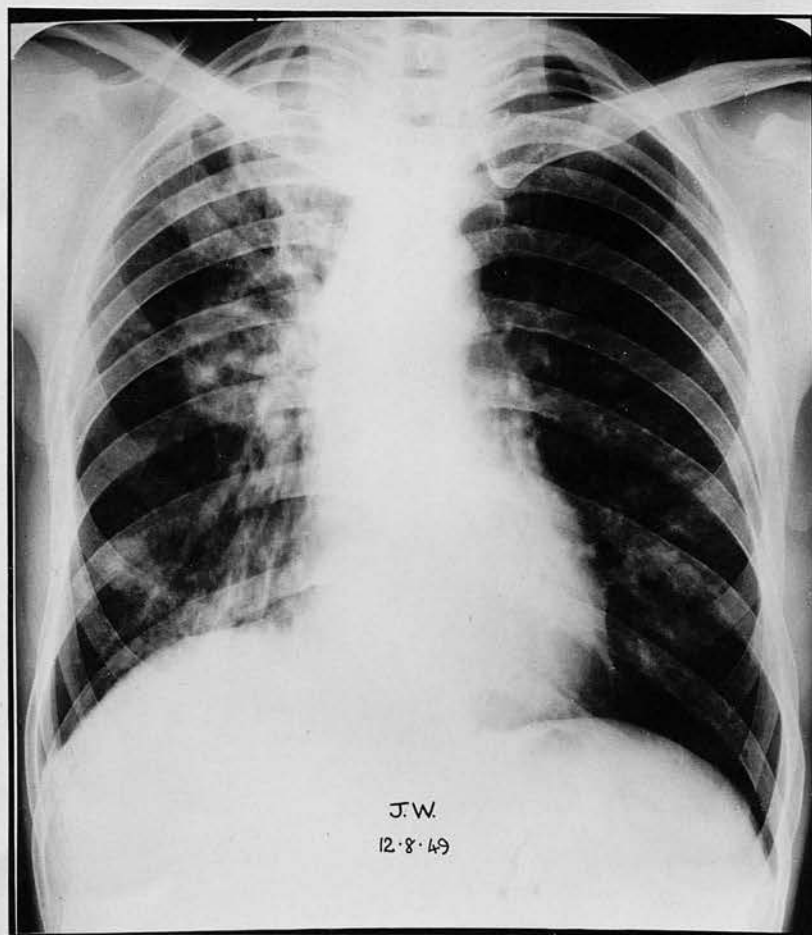


FIG. 60. J.W.

After 3 mths.

P.A.S.

Case 21. N.C. A young woman aged 21 whose history illustrates the insidious onset which may sometimes occur with active tuberculosis in a young adult. A cough, unproductive at first, had been present for about six months followed later by the gradual onset of lassitude, night sweats and loss of weight.

When she was first seen she was pale, thin and toxic looking. Weight was 7 st. 10 lbs. and widespread activity could be heard on auscultation. The X-ray picture (Fig. 61) revealed bilateral active tuberculosis involving the apex, upper and mid zones of both lungs. No definite excavation could be seen but there were areas suggestive of early softening in both lungs.

Tubercle bacilli were present in moderate numbers in the sputum.

#### Treatment

The only treatment possible with this patient was bed rest supplemented by some form of chemotherapy. It was decided to institute treatment with P.A.S. without delay.

Length of course: At the time of writing 12 weeks' treatment had been given, and the course was continuing.

Dosage: 25 gm. daily

Signs of intolerance: nil

Average blood level: 7 mgm. per cent.

#### Progress/

### Progress under treatment

Steady improvement in general condition, temperature disturbance (range 99° - 101°F) settled to within normal limits during the first two weeks of treatment. There was a steady gain in weight amounting in the 12 weeks to 4 lbs. and the B.S.R. readings fell from 66 to 13.

Tubercle bacilli have disappeared from the sputum and gastric washings. The result of culture is not yet to hand.

Radiological changes: Fig. 62 illustrates the appearances at the end of two months' treatment. Considerable resolution has occurred in all areas although the disease is still highly active and extensive.

### Summary

Improvement in general condition.

Improvement in radiological appearances.

### Conclusions

The response to treatment in this patient has been extremely promising and is well without the range which could be expected on bed rest alone. Whether or not permanent benefit will accrue it is impossible to say at this stage.

### Prognosis

Unchanged as yet.



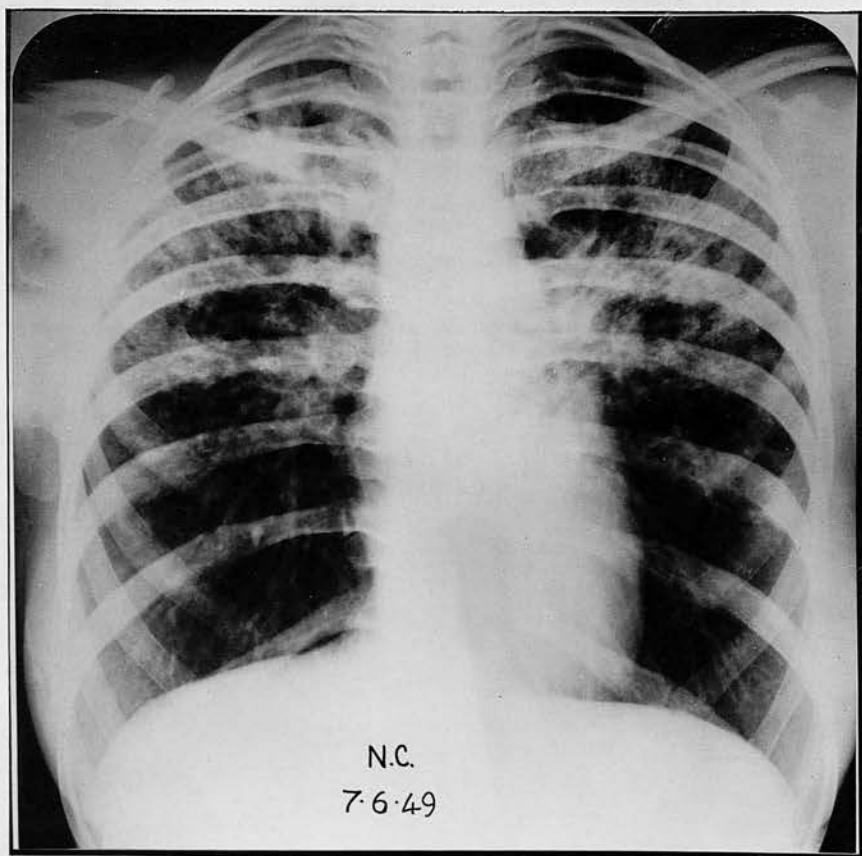


FIG. 61. N.C.  
On admission.

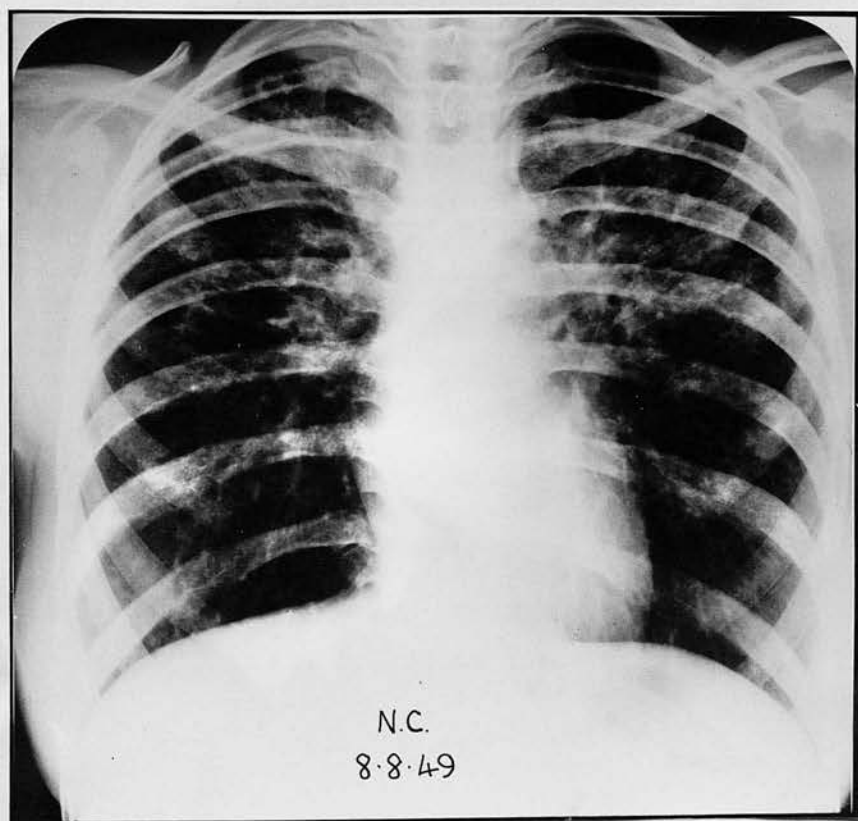


FIG. 62. N.C.  
After 2 mths.  
P.A.S.

Chapter 8ANALYSIS OF RESULTS

A careful assessment of the progress made by each patient as a result of treatment, including changes in general condition and radiological appearances, was made. The following figures were obtained:

1. Pronounced improvement (+++) 2 cases, 10%.
2. Considerable improvement (++) 4 cases, 20%.
3. Appreciable improvement (+) -- 11 cases, 55%.
4. Condition stationary (<sup>+</sup><sub>-</sub>) ----- 2 cases, 10%.
5. Condition deteriorated (-) ---- 1 case, 5%.

One case in each of the first two groups was given a supplementary course of streptomycin during treatment with P.A.S.

Prognosis

In each of the six cases composing the first two groups above, i.e. in 30% of the patients in this study, the prognosis was considered to be materially improved by treatment.

The prognosis of two patients in group 3 was considered to be slightly improved while in the remainder (60%) the prognosis was not altered as a result of treatment.

A more detailed analysis of the results of treatment is contained in the following four tables.

TABLE 10/

TABLE 10.

Progress of 20 patients under treatment with P.A.S.  
related to the extent of the disease at the start of  
treatment.

Extent of Disease	Progress under Treatment					TOTAL
	+++	++	+	$\pm$	- *	
Early	0	0	2	0	0	2
Inter- mediate	0	1	3	0	0	4
Advanced	2	3	6	2	1	14
TOTAL	2 10%	4 20%	11 55%	2 10%	1 5%	20 100%

\* For explanation of classification see p. 168.

TABLE 11/

TABLE 11.

Progress under treatment of 20 patients related to  
the length of course.

Length of Course (weeks)	Progress under Treatment					TOTAL
	+++	++	+	$\pm$	-	
12	0	0	5	0	0	5
15	0	0	0	1	0	1
17	0	1	0	0	0	1
18	0	1	1	0	0	2
20	0	0	1	0	1	2
22	0	2	3	0	0	5
24	2	0	1	1	0	4
TOTAL	2	4	11	2	1	20

TABLE 12/

TABLE 12.

Progress under treatment of 20 patients related to the daily dose of P.A.S.

Daily Dose (gms.)	Progress under Treatment					TOTAL
	+++	++	+	$\pm$	-	
15	0	0	3	0	0	3
20	0	0	0	1	0	1
24 and 25	2	4	8	1	1	16
TOTAL	2	4	11	2	1	20

TABLE 13.

Progress under treatment of 20 patients related to the average blood level of P.A.S. obtained throughout the course.

Av. Blood Level (mgm%)	Progress under Treatment					TOTAL
	+++	++	+	$\pm$	-	
3-3.9	0	1	2	0	0	3
4-4.9	1	1	1	1	0	4
5-5.9	0	0	4	1	1	6
6-7	1	2	4	0	0	7
TOTAL	2	4	11	2	1	20

The numbers in individual groups are necessarily small/



small and, without adequate controls, conclusions must be merely tentative.

Nevertheless a scrutiny of the tables does indicate that the results of treatment are not entirely dependent on the length of the course although they tend to improve as the number of weeks increases. Similarly they are not dependent on the blood level of the drug obtained. A study of Table 12 does, however, appear to show that the higher daily dosage (24 gm. and 25 gm.) is advantageous, although, it has to be remembered, that the intended dose for each patient was 25 gm. and that signs of intolerance necessitated the reduction of the dose.

## DISCUSSION

### Properties of a chemotherapeutic agent

Feldman and Hinshaw (1944) who have amassed a vast experience in elucidating the complex and differing problems afforded by any investigation into the efficacy of a chemotherapeutic agent in tuberculosis, have laid down properties to be possessed by any drug proposed for clinical study in tuberculosis.

These are that:

- 1) It should have the ability to restrain, arrest or overcome well established tuberculosis in experimental animals, which investigation should be adequately controlled.
- 2) Experimental animals should be able to tolerate continued administration of the drug in effective doses over/

over periods of many months without damage to vital organs.

3) Preliminary trial of the drug in human beings should have determined that hazards of treatment are absent or are sufficiently slight when compared with the anticipated therapeutic result to justify any discomfort or risk entailed, and that sufficient should be known of the pharmacology to ensure appreciable concentration in the blood, and to prevent or limit undesirable side effects.

The first two of these provisos regarding P.A.S. have been demonstrated amongst others by Feldman and Hinshaw, themselves, (Feldman, Karlson and Hinshaw, 1947). Knowledge as to the third has gradually increased since the pioneer work by Lehmann, and P.A.S. has proved to be a drug with negligible toxicity.

#### Present study

Reasons for the lack of adequate controls in this series of cases have already been discussed but several features of the investigation are worthy of comment.

#### Beneficial effects of P.A.S.

From a study of the literature, which is gradually accumulating regarding the use of this drug, it is soon evident that there are one or two features of the effect of P.A.S. in human tuberculosis which, despite the varied nature of the cases, are constantly recurring. Results in this investigation have/

have been similar and it would be interesting to note one or two.

1) Improvement in general condition. Erdei and Snell (1948) remarked upon this finding as a striking feature of the effect of P.A.S. on their cases. Similarly a large number of the patients in this study illustrated this improvement to varying degrees.

2) Reduction in temperature. Although only a small number of the patients were subject to temperature disturbance before treatment, in those in whom it was manifest, the action of the drug in restoring it to within normal limits appeared unequivocal. The prominence of this feature in the trial reported by Erdei was of such a nature as to cause him to attribute to the drug antipyretic properties.

3) Fall in B.S.R. The universal finding of an initial fall in the blood sedimentation rate, maintained in some, and followed in others by a subsequent slow rise, is a feature of the effect of the drug which cannot adequately be explained but may be related in some way to the property of the drug which causes the reduction in temperature.

4) Bacteriology of sputum. Conversion of sputum from positive to negative in nine cases, with reduction in the number of bacilli in others may be related, in part at least, to the direct action of the drug on the bacillus. It is an important finding/

ing and may prove a valuable property of the drug. It remains to be demonstrated how permanent a feature of each case this is.

5) Radiological evidence of resolution of foci. A universal finding in this series of cases was the resolution of the exudative, infiltrative type of lesion. A very slight degree only, occurred in some, while in others considerable resolution was recorded. In no case was resolution complete but nevertheless it seemed to be of a sufficiently striking nature to allow the conclusion that one of the properties of P.A.S. is to promote the resolution of such foci.

6) Action on cavities. Considerable reduction in size of cavities took place in many cases. In some this was maintained throughout treatment; in some complete closure was recorded but in others return to the original size took place. A similar finding was recorded by Dempsey and Logg and suggests that, in some cases at least, beneficial effect of the drug on disease of the draining bronchus altered the mechanics of the cavity and thus produced reduction in size.

Fibrosis was not a striking change in this series although Erdei (1943) has recorded it as being a prominent feature and Youmans (1947) suggested that the beneficial action of P.A.S. was due to the change of the host's response from necrotic exudative to fibrotic proliferative.

Results of treatment/



### Results of treatment

The vital question to be answered when investigating the effect of any therapeutic agent is whether, as a result of treatment, the prognosis is altered favourably and permanently. It will be remembered that in 30% of cases the prognosis was altered favourably and, it is hoped, permanently; in 10% the prognosis was slightly improved and in the remaining 60% the prognosis was unaltered. This latter group does however include two patients in whom the prognosis at the start of treatment was not serious.

These figures indicate, therefore, that, despite a number of beneficial effects which without doubt are attributable to the drug, the drug is not powerful enough to alter permanently the course of the disease when extensive lesions exist.

Available evidence would seem to show that the drug is bacteriostatic in its properties; that, therefore, a cessation of retrogression, albeit temporary, can be expected; but that much must depend on the individual powers of resistance possessed by the patient in determining the outcome of the disease.

### Pointers for the future.

Although P.A.S. has not, as evidenced by the results of this series of cases, proved to be a chemotherapeutic agent of the first importance it would appear to be a drug with tuberculostatic powers/



powers, and possesses the advantages of ease of administration and lack of toxicity. It would, therefore, be advantageous to examine, in the light of experience gained, how best to utilise it in the future.

1. Type of case: The resolution described in the exudative lesions present in the patients composing this study conforms with the belief of Feldman and Hinshaw that chemotherapeutic agents are most likely to be beneficial in this type of disease, as it most resembles the type of lesion found in experimental animals on which the efficacy of the drug has been proved. In the case of P.A.S., too, Alin and Helander (1948) have described its deposition in the elastic tissue of the normal lung. We should, therefore, expect a greater effect from the drug in disease which has not affected the normal, anatomical structure of the lung.

It will be remembered, however, that a feature of the improvement shown was reduction in size of cavities. It would seem, therefore, that the drug might also be of use in cases in which small thin-walled cavities were present and gross destruction of lung tissue had not taken place.

Use of the drug could also be made in more extensive disease in preparing the patient for further collapse therapy or to control a recent exudative spread from more long standing disease. The treatment of the seriously advanced case is not yet/

yet solved as the use of P.A.S. to stay the downward progress of the disease would merely result in fresh problems regarding the management of the case when the treatment was stopped.

Whether the direct action of the drug on the tubercle bacillus is an indication for its use in reducing the infectivity of a case before admission to hospital is still a matter for discussion.

As this investigation was concerned only with pulmonary tuberculosis it is not proposed to discuss the use of P.A.S. in other forms of tuberculosis.

2. Length of course: The beneficial effect of P.A.S. is manifest quickly and to the greatest degree in the first three months of treatment.

Horne (1949) noted an interesting return to the original rate, after an initial lengthening, in the culture period of the sputum of patients who had been under treatment for 8-10 weeks, and suggested that this might be due to the development of a P.A.S. resistant strain of organism. Drug fastness has not however yet been demonstrated (Lehmann, 1947, Erdei, 1948) and it would seem, therefore, in the absence of serious toxic effects, that the only limit to the length of the course is the patient's continued cooperation.

Consequently, after the initial period of improvement, if manifest, continuation with P.A.S. is recommended, principally, in order that its bacteriostatic effect may assist the patient to consolidate/

consolidate the improvement which has taken place. A course of five to six months is therefore advised.

3. Dosage: Although the evidence regarding the effect of dosage and resultant blood levels is not complete it is recommended that the higher dose of 25 gm. daily, if within the limit of the patient's tolerance, should be given. Until further evidence becomes available also, it would seem that the omission of the night doses and one day's rest from the drug in seven, despite the fall in blood concentration which occurs at those times, is important because of its stimulating effect on the patient's mental condition.

The rapid excretion of P.A.S. by the kidneys, necessitating as it does frequent administration, constitutes a serious drawback of P.A.S. therapy. Some method of delaying this excretion and thus permitting fewer, more widely spaced doses would greatly promote the patient's comfort. In all probability, a higher and more prolonged blood concentration would also be obtained. It is understood (personal communication) that work is proceeding to investigate the possibility of the use of caronamide for this purpose.

4. Combination with streptomycin: Vennesland et al (1948) showed that the in vitro effect of a combination of P.A.S. and streptomycin on the growth of tubercle bacilli was greater than the addition of the/

the result of each when acting alone. Whether or not the striking result obtained when the two drugs were combined in Case 9 of this series is an example of this phenomenon acting in human tuberculosis is a matter for conjecture.

It has also been shown that P.A.S. is effective in vitro against streptomycin resistant strains of the bacillus (Vennesland et al, 1948), and that, as well as the above, combination with P.A.S. will retard the development of a streptomycin resistant organism both in vitro (Graessle and Pietrowski, 1949) and in vivo (Karlson et al, 1949).

These facts indicate a possibly fruitful field for future investigation.

#### CONCLUSIONS

a) Para-aminosalicylic acid in the form of the sodium para-aminosalicylate salt exerts a beneficial effect in pulmonary tuberculosis.

b) This effect is greatest in exudative lesions and consists in the promotion of resolution of these lesions.

c) The effect is not of a sufficient degree to alter permanently the course of the disease in the majority of advanced cases.

d) The greatest amount of benefit from P.A.S. occurs in the first three months of treatment.

e) P.A.S. is instrumental in reducing the size of certain cavities. The exact nature of the change which takes place is not known.

f)/



f) P.A.S. produces an improvement in the general condition of patients suffering from pulmonary tuberculosis.

g) A marked initial fall occurs in the blood sedimentation rate following the administration of P.A.S. in patients suffering from pulmonary tuberculosis.

h) No serious or irreversible toxic effects ensue from the administration of the drug.

i) Within the limits of this uncontrolled study, the effect of P.A.S. is not strikingly related to the length of course, or level of blood concentration obtained.

j) Para-aminosalicylic acid is not a therapeutic agent of the first importance in the treatment of pulmonary tuberculosis, and its value in the future may lie in combination with streptomycin.

#### ACKNOWLEDGEMENTS

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